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A peracid sterilant formulation was developed to the Army's performance requirements for a powdered cold sterilant for field use as a replacement for glutaraldehyde. The active biocidal agent, peracetic acid, is generated in situ and the working life may be up to eight hours with reuse at 20°C. AOAC carrier testing showed the formulation to be sporicidal within 20 minutes at its minimum effective concentration and to pass the AOAC Use-Dilution Test, the AOAC Fungicidal Test, EPA Tuberculocidal Test and virucidal tests at a 10 minute contact time or less following simulated reuse after eight hours. The use-dilution is safe per toxicological evaluations. Common materials of construction of medical devices were shown to be compatible and the sterilant can be effectively rinsed from devices. In-use (clinical) studies were successfully conducted at two medical centers. The formulation is chemically stable in shelf; however, starting from about 9 months in storage the efficacy (B. subtilis D-value testing) was significantly reduced. While a number of medical devices were shown to be sterilizable, the solution was unable to consistently sterilize others including flexible lumened scopes. Newer lots produced showed inferior performance. Due to these concerns, STERIS convened a meeting of experts to discuss these issues. The resultant work schedule and testing did not reveal the cause of the unfavorable microbiological results. STERIS has abandoned the development of this formulation and will not make a regulatory filing. STERIS continued its research and development efforts on a reformulated product and has shown in preliminary studies as discussed herein its microbicidal efficacy.

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PRINCIPAL INVESTIGATOR: PAUL S. MALCHESKY, D.Eng.

CONTRACTING ORGANIZATION: STERIS Corporation
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Fort Detrick, Frederick, MC 21702-5012

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FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

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____ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

_____ For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

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Paul S. Wakelesky — 10/31/96

PI - Signature — Date

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INTRODUCTION (BACKGROUND TO PHASE II WORK)

Field medical use of many surgical instruments, including delicate instruments such as endoscopes, requires cold sterilization, cold being defined as a temperature range between ambient (about 20°C) and moderately elevated (up to 60°). Because of the hazards in transporting and using ethylene oxide, it has been eliminated from field medical use. Glutaraldehyde, a disinfectant which has many undesirable characteristics involving logistics support, user safety and product effectiveness, is currently being used as a replacement. Required is a safe and effective dry powder sterilant that can be added to water to effect the cold sterilization of instruments. It should be packaged as an inert powder that can be safely and efficiently transported and stored and the waste solution should not pose a toxic hazard to users.

In response to the Department of Defense FY 1991 Small Business Innovation Research (SBIR) Program solicitation 91.2, STERIS Corporation submitted a Phase I proposal for The Development of a Cold Sterilant for Field Medical Use. This proposal was approved and a contract (DAMD 17-93-C-2049) was awarded in March of 1992. The objective of Phase I was to develop one or more powdered cold chemical sterilants for field medical use and demonstrate that these products: (1) were effective against bacterial endospores and viral, bacterial and fungal contaminants, and (2) otherwise meet medical field use sterilization requirements set forth by the United States Army Medical Research and Materiel Command (USAMRMC).

The Phase I proposal contained a list of Performance Requirements for a Powdered Cold Sterilant which had been developed as a result of discussions between STERIS and USAMRMC personnel during a demonstration of the STERIS SYSTEM 1™ Sterilant Processor at Fort Detrick in October of 1990. The consensus product performance specifications for a powdered sterilant which can be added to water to effect the cold sterilization of surgical instruments including delicate units such as endoscopes are as follows:

1. Efficacy: The sterilant will consistently kill bacterial spores as defined by : (a) D-values, and (b) minimum AOAC kill times.

2. Safety: The sterilant will not be toxic to users and the use dilution can be safely disposed of without special handling.

3. Time: Time required to effect sterilization is to be 20 minutes.

4. Temperature: The temperature at which sterilant (powdered chemical dissolved in water) will affect sterilization was originally set at 25°C. Based upon discussion between the U.S. Army, STERIS and FDA personnel on September 2, 1993, the use temperature was adjusted down to 20°C.

5. Concentration: The parts of dry sterilant per one gallon quantity of water will be easily dissolvable in water at ambient temperature.

6. Multiple Uses: The solution should be stable for reuse for a minimum of eight hours.

7. Material Compatibility: Sterilant will not corrode or otherwise harm materials or adhesives used in instruments to be sterilized.

8. Water Quality: To military specification for potable water.

9. Stability During Transport: Stability is defined according to Mil STD 810-D.

10. Shelf Life of Powdered Chemical: Length of time the packaged chemical can be stored and retain effectiveness will be greater than one year at accelerated conditions specified by the EPA.

11. Shelf Life of Sterilant Solution: Length of time sterilant solution will remain efficacious will be 8 hours.

12. Packaging: Single use unit packaging, accessible without special equipment (for example a rip and pour pouch) and meeting transport requirements (such as air drop).

STERIS's Phase I work indicated that peracid generating compounds could meet the USAMRMC need for a cold sterilizing dry powder sterilant capable of replacing glutaraldehyde solutions. During the Phase I research an extensive literature search conducted identified a number of potential compounds that could be used to produce a cold sterilant active at the USAMRMC specified temperature of 25°C. These compounds and one that STERIS had previously developed for use at 50°C and registered with the U.S. Environmental Protection Agency were screened for their sterilizing potential at 25°C. The research showed that a modified version of the STERIS formulation (Formulation A) designed for use at 50°C was also the most efficacious at 25°C with one possible exception (Formulation B) that may offer packaging development advantages.

It was concluded from Phase I research that an all dry powder sterilant meeting the USAMRMC requirements could be developed using either Formulation A or B. It was proposed in Phase II to further test and define the relative merits of Formulation A and B and to select the best for full development into a finished product meeting USAMRMC specifications.

The objectives for Phase II were to: (1) select through further testing one of two sterilant formulations developed through Phase I, (2) develop packaging for the selected sterilant to assure safe and efficient transportation and storage, (3) further demonstrate sterilant safety

and efficacy by obtaining EPA regulatory approval and FDA clearance to market and (4) deliver to USAMRMC prototype packaged sterilants (with appropriate labeling and use instructions and biological and chemical indicators) to permit the USAMRMC to conduct initial field tests following completion of Phase II.

AWARD OF PHASE II CONTRACT

STERIS was granted a Phase II contract for the "Development of a Cold Sterilant for Field Medical Use" in the spring of 1993 with Dr. Raymond C. Kralovic as the Principal Investigator. Work under this contract commenced in April of 1993. With a change of personnel at STERIS, Paul S. Malchesky, D.Eng. replaced Dr. Kralovic as the Principal Investigator in March of 1994.

REGULATORY TESTING REQUIREMENT

At the outset it was recognized that technical challenges existed in developing a sterilant formulation that would meet the requirements outlined above. In addition, the primary Phase II technical challenges relate to fully satisfying the demands of the regulatory agencies. In January, 1992[1] the FDA revised its guidelines for 510(k) submissions for liquid chemical germicides and did again in April, 1995.[2] It is these guidelines, in addition to those published by the EPA, that were used in developing the test plans and conducting the evaluations on the sterilants developed in this program.

A project plan was agreed to by the Army and STERIS in March of 1993. This plan called for regulatory submissions. On June 4, 1993 the EPA and FDA executed a Memorandum of Understanding (MOU) which described a change in regulatory responsibilities of the respective agencies concerning liquid chemical germicides intended for use on medical devices. To best understand the impact of the MOU, representatives of STERIS and the Army met with the FDA on September 2, 1993 to discuss its project. It was confirmed in this meeting that the FDA had primary responsibility for review of efficacy and safety data, although such data should be submitted to both agencies. A review of the test plan confirmed the testing requirements and the need to add clinical trials. Also during the discussions of the performance requirements, FDA questioned whether the 25°C temperature criterion represented actual use requirements. It was noted that this was a contract performance requirement provided by USAMRMC. It was felt at that time that the FDA would pursue this question when it reviews the results of clinical trials. Subsequently, STERIS decided to conduct testing at 20°C.

TEST RESULTS

STERIS over the course of this program has submitted to the Army progress reports (typically on a quarterly basis) that summarized its findings. In an effort to best summarize these results they will be discussed under the following topic headlines.

- Formulation Selection
- Sporicidal Testing
- Other Microorganisms Studied
- Physical/Chemical Properties
- Shelf Life/Packaging
- Toxicology
- Materials Compatibility
- Effect of Precleaner Residuals
- Medical Device Testing
- In-Use (Clinical) Testing
- Chemical Indicator Development
- Biological Indicator

FORMULATION SELECTION

In the early months of the Phase II contract additional studies were conducted on the Formulations A (uses acetylsalicylic acid as the acetyl donor) and B (uses tetraacetyl ethylene diamine as the acetyl donor) developed in Phase I. Formulation A is based on the patented dry formulation denoted as STERIS 20D. Formulations A and B contain buffering agents, sequestrants, surfactants and perborate (H_2O_2 generator) which reacts with the acetyl donor to produce peracetic acid. Both chemical and sporicidal testing were carried out. Although Formulation B appeared to be equivalent to Formulation A when freshly prepared, it was found to be inferior to Formulation A when tested for sporicidal activity (D-value) 8 hours after activation, Table 1.

The reason for this loss in sporicidal activity is because Formulation B generates peracetic acid (PAA) more rapidly and reaches a higher peak concentration than does Formulation A, but after about 3 hours the PAA concentration is less than that of Formulation A. It was demonstrated during Phase I studies that PAA stability and generation rates are dependent on the initial pH of the reaction. A higher pH favors a more rapid generation of PAA, especially from Formulation A, and a lower pH increases the stability of peracetic acid but

slows down the rate at which it is generated.[3] It was found that the optimal conditions (i.e., rapid PAA generation and stability with time at 25°C) could be obtained by using the combination of the two acetyl donors such that approximately one-half of the PAA is generated by each of the precursors. As shown in Figure 1, the combination of the two acetyl donors (Formulation C) produces the most rapid generation of PAA. The combination of acetyl donors also has the ability to maintain this concentration for 8 hours or more after activation.

Table 1 Sporicidal Activity Of Formulations A & B At 25°C For Bacillus Subtilis Spores

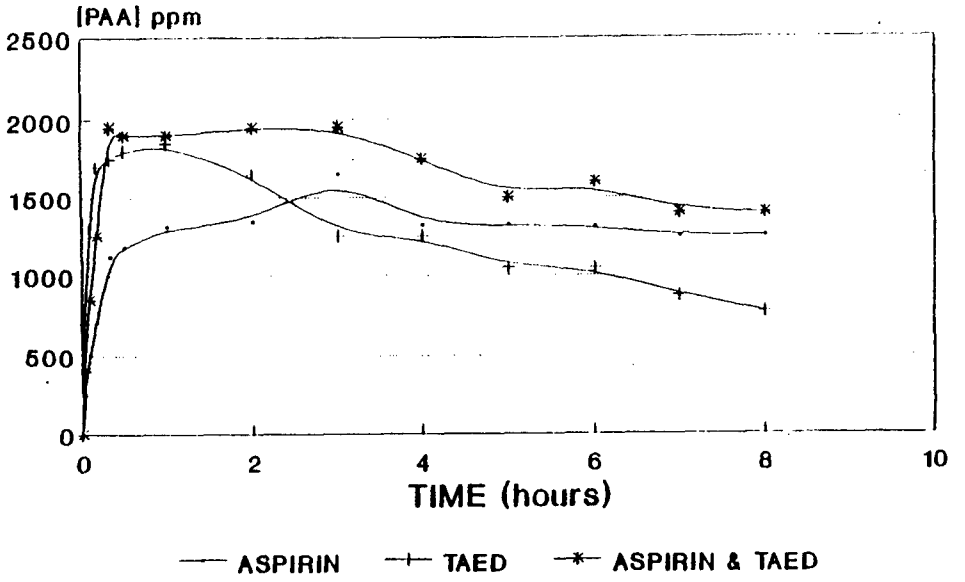
		Formulation A		Formulation B	
*Time (hr)	Exp.	**% Kill	D-Value (sec)	**% Kill	D-Value (sec)
0.5	1	99.9992	58.9	99.9399	93.10
	2	99.9828	79.9	99.9996	55.10
	3	99.9998	51.4	99.9996	55.30
		Mean = 63.3 ± 12.0		Mean = 67.80 ±17.9	

		Formulation A		Formulation B	
*Time (hr)	Exp.	**% Kill	D-Value (sec)	**% Kill	D-Value (sec)
8.5	1	99.9998	51.4	80.2272	426.20
	2	99.9978	64.2	84.9242	365.10
	3	99.9996	55.2	81.7424	405.21
		Mean = 56.9 ± 5.4		Mean = 398.80 ±25.3	

* Time After Activation

** Initial Population was 1.1×10^8 cfu/ml

Figure 1 Generation Of Peracetic Acid (PAA) By The Acetyl Donors



Preliminary studies of the corrosivitity of Formulation C indicated it is somewhat more corrosive to brass than other formulations developed by STERIS. Studies were carried out to determine what modifications needed to be made. These modifications were then made to the formulation. A patent disclosure on this formulation has been filed. A confidential statement of formulation was developed for Formulation C which is referred to as STERIS 20C or 20C. The biocidal chemical agent of STERIS 20C Sterilant is peracetic acid that is generated through the reaction of sodium perborate monohydrate (the oxygen donor) with acetylsalicylic acid (aspirin), and carboxymethyl-cellulose-encapsulated tetracetylethylenediamine (TAED). The reaction proceeds beginning with the generation of hydrogen peroxide from the hydrolysis of sodium perborate. Hydrogen peroxide and its reactive species react with the acetyl groups donated by the aspirin and TAED to form peracetic acid. The breakdown products of aspirin and TAED are salicylic acid and diacetylethylene diamine (DAED).

In addition to sodium perborate, the builders formulation of STERIS 20C contains sequestrants, buffering agents, a surfactant, a defoamer, and anticorrosive agents. The sequestrants act as strong chelating agents that will react with metallic ions in solution to form soluble complexes. The buffering system is used to create an initial pH of 8.0 - 8.5, which is favorable for PAA generation. This buffering system will also increase the stability of the generated PAA by stabilizing the pH at 7.0 - 7.5. A defoamer is added to decrease the foam created during preparation. A surfactant is utilized to reduce the surface tension of the solution and allow more penetratability of the sterilant to device surfaces. A metal anticorrosive agent is employed to increase the compatibility of metals with the sterilant.

Initial batches of the sterilant for testing were produced in kitchen size blenders. Subsequently two blends were made in a manufacturing scale VEE-blender and were used in shelf life stability testing studies.

PREPARATION OF STERILANT

STERIS 20C sterilant is designed to be used in a manual soak technique at room temperature for liquid immersible reusable medical devices with an exposure time dependent upon the classification of the device as critical or semicritical. Devices classified as critical require sterilization and would require a 20 minute exposure time to STERIS 20C. Semicritical devices may require lower exposure times.

In the initial months of the program, studies were initiated to determine the powdered sterilant dissolution method and the time to activate the solution prior to use (total activation time of 30 minutes. It was determined that 5 minutes of either mechanical or manual stirring was required.

After repeating these studies (using the same mixing methods) for the purpose of defining the conditions under which clinical trials would be carried out, it was concluded that the laboratory mixing methods were feasible but not user friendly. Other variable use condition factors effecting activation were identified including mixing water temperature, mixing time, and mixing container type.

To improve sterilant dissolution time various techniques and/or methods were investigated including the use of elevated temperatures, chemical particle size reduction and mixing methods. In particular water at elevated temperatures was most effective. Originally, dissolution of the sterilant powders was not considered to be an issue as a much shorter dissolution time is claimed by the supplier of the chemical component (TAED) which has proven to be difficult to dissolve. Contamination of this component was indicated in some testing, but this was not proven to be the problem.

The sterilant solution can be prepared using one of two techniques: a shaking method preparation using warm water or a continuous mechanical mixing method using room temperature water.

SPORICIDAL TESTING

Initial sporicidal testing at use dilution with Formulation C, 20C, was carried out per the AOAC sporicidal test. This test requires sterilization of 180 carriers of each of the four types (suture loops and penicylinders inoculated with Clostridium sporogenes and Bacillus subtilis spores) with no sterilization failures using 3 lots of sterilant, one of which is 60 days old from the date of manufacturing. In addition to these requirements, since the sterilant is to be reusable for up to 8 hours, testing was conducted per a reuse protocol MS 93-002 (see Appendix).

As reported in STERIS Technical Report T 94-004 (see Appendix) one hundred and eighty (180)

of each of the four types of carriers (209 of 210 C. sporogenes suture loops) were sterile when exposed for twenty minutes to the sterilant, after completion of 21 days incubation, heat shocking and three days additional incubation. One Clostridium suture loop was initially recorded as positive after heat shocking at the twenty-one day point, consequently an additional set of thirty C. sporogenes suture loops were exposed to the sterilant for the required twenty minutes. All these were shown to be sterile. The positive control data showed that the builders without the acetyl donors do not sterilize any of the four carrier types.

Testing was also completed on carriers per the reuse protocol after 8 hours with the use dilution sterilant at 20°C. As outlined in STERIS Technical Report T94-005 (see Appendix) all 180 carriers of each four types tested with the stressed sterilant solution at 8 hours of use were sterile when exposed for twenty minutes to the sterilant at completion of 21 incubation, heat shocking and three days additional incubation at 37°C. The positive control data indicated that the builders without the precursors did not sterilize any of the four carrier types.

To determine the efficacy of the stressed sterilant solution (per protocol MS93-002) at the starting time and after 8 hours independent of the peracetic acid concentration, D-value testing was carried out (STERIS Technical Report T94-009, see Appendix). Testing showed that there is no significant difference in the D-values for B. subtilis or C. sporogenes at the start of or end of the sterilant reuse time. This study also demonstrated that the B. subtilis spores are more resistant than C. sporogenes spores.

As noted in the Food and Drug Administration's document for Premarket Notification Submission for Liquid Chemical Germicides dated October 26, 1995[2] it is required that microbial effectiveness be shown under the worst case composition for a sterilant or the minimum effective concentration of a sterilant be determined. The minimum effective concentration determination was studied for STERIS 20C sterilant by the end point sporicidal D-value method using Bacillus subtilis ATCC 19659. B. subtilis was chosen based on the results of previous end point D-value testing which indicated that for peracetic acid based liquid sterilants it has a longer D-value and end-point time than Clostridium sporogenes in 1000 ppm ($CaCO_3$) hard water with 5% bovine serum. The data for STERIS 20C sterilant suggests a minimum effective concentration of 800 $\pm$ 50 ppm at 20°C which gives a 12 D-value of less than eight minutes which is less than the twenty minute exposure time for sterilization (STERIS Technical Report T94-010.1, see Appendix).

The minimum effective concentration (MEC) for efficacy was studied using AOAC sporicidal carriers in hard water at 1000 ppm as $CaCO_3$, a concentration higher than recommended by the FDA Guidance, with 5% bovine serum (STERIS Technical Report T94-015.1, see Appendix). All 720 porcelain penicylinder and Dacron suture loop carriers of B. subtilis and C. sporogenes studied as per the AOAC methods were shown to be sterile at 20 minutes at 20°C. These results confirm the sporicidal efficacy of STERIS 20C at 800 $\pm$ 50 ppm peracetic acid concentration at 20°C under reuse conditions at worst case composition of the sterilant with an inorganic and organic load. End point determination indicated a maximum kill time equal to or less than 12

minutes for Bacillus subtilis ATCC 19659 carriers and equal to or less than 2 minutes for Clostridium sporogenes ATCC 3584 carriers. The maximum end point times were much less than the recommended twenty minutes sterilization time.

The sporicidal efficacy of the sterilant to inoculated carriers was confirmed by testing carried out by Gibraltar Laboratories (Report G-79228, see Appendix). Sixty (60) carriers of Clostridium sporogenes suture loops and penicylinders and 60 Bacillus subtilis suture loops and penicylinders were tested using two sterilant lots, P-1 and P-2, each of which was nine months old. All 120 carriers were sterile after 20 minutes exposure time. The maximum end point for B. subtilis penicylinders and suture loops were $\leq$ 4 minutes. The maximum end point for C. sporogenes penicylinders was $\geq$ 4 minutes which was the last time point before the 20 minute exposure time. For C. sporogenes suture loops the maximum end point was $\leq$ 4 minutes.

Since the recommended user conditions for STERIS 20C is to dissolve the powders in warm tap water, the microbiological efficacy was determined for temperatures from 20°C to 50°C in increments of 10°C for a peracetic acid concentration of 1000-1100 ppm. The end point sporicidal D values using B. subtilis ATCC 19659 were 20.53 seconds at 20°C, 17.06 seconds at 30°C, 7.32 seconds at 40°C and 2.38 seconds at 50°C (STERIS Technical Report T94-017.1, see Appendix). Thus, the D-value times decreased as the temperature was increased. The 12-D value times were all less than the recommended twenty minute sterilant exposure time recommended for use with the sterilant.

To assess the sterilant's kill rate STERIS contracted with Gibraltar Laboratories to conduct D-value tests on a variety of organisms. Table 2 reports the organisms tested and their D-value as reported in Gibraltar reports G-79475, G-78632, G-79227, G-78100, G-78100.1, G-79867 and G-79769 (see Appendix for reports) .

Table 2 D-Values For STERIS 20C

ORGANISM		**D-VALUE** (sec)
S. aureus	ATCC 6538	4.33
S. choleraesuis	ATCC 10708	4.70
P. aeruginosa	ATCC 15442	5.30
Herpes Simplex Type 2	ATCC VR734	6.38
C. sporogenes	ATCC 3584	8.82
T. mentagrophytes	ATCC 9533	12.00
B. coagulans	ATCC 7050	13.14
B. circulans	ATCC 4513	24.96
B. subtilis	ATCC 19659	25.74
B. stearothermophilus	ATCC 7953	25.74
B. cereus	ATCC 11778	32.76
B. subtilis var. *niger*	ATCC 9372	40.32*
B. pumilus	ATCC 27142	66.00
M. bovis	ATCC 35743	96.30*
Poliovirus 2 (dried film)	ATCC 1002	<144.00*
Poliovirus 2 (suspension)	ATCC 1002	300.00*

*Testing repeated, see results below.

Because of the higher D-values than expected for poliovirus and Mycobacterium additional testing was commissioned with this test laboratory. In the study of poliovirus in a dry film the D-value was found to be <144 seconds. In the follow-up study additional contact times were selected to better characterize the D-value. The D-value was determined to be approximately 24 seconds (Gibraltar Report G-87871, see Appendix) showing that STERIS 20C is virucidal within the recommended exposure time for use of the product.

In testing of poliovirus the D-values were longer when the organism was in a suspension than when it was a dry film. In studies of the suspension it was shown that the resultant D-value is related to the methodology. In studies without sonication of the viral suspension the D-values were about 5 minutes. With sonication for four minutes immediately prior to viral suspension addition to the sterilant solution, the D-value was 4 minutes. Repeat testing showed a D-value of 3.8 minutes (Gibraltar Report G-78100, see Appendix). Thus sonication lowered the D-value but even still these D-values are higher than that for the dry film. In additional studies conducted by Gibraltar (Gibraltar Report G-87755, see Appendix) it was demonstrated that increasing sonication time from 4 to 8 minutes further reduced the D-value to approximately 90 seconds. The results suggest that poliovirus is initially present in an agglutinated, complex state which is recalcitrant to the sterilant. Sonication appears to alter the viral state making the virus susceptible to inactivation. This study demonstrates that the methodology of testing is important and that while suspension testing of the poliovirus gives a higher D-value than for the dry film; in application of the sterilant, sufficient kill of poliovirus can be achieved at the recommended use time of 20 minutes.

In the conduct of the poliovirus D-value studies there was a concern that the media and reagents and the poliovirus suspension itself may be inhibitory to the sterilant's activity. To assess this hypothesis the kill of B. subtilis spores prepared in the virus dry films was assessed by D-value studies. The D-value for B. subtilis was shown to be $\leq$ 13 seconds for the sterilant lot over 21 months old (Gibraltar Report G-87597, see Appendix). This study showed that neither the poliovirus nor the media significantly impair the sporicidal ability of STERIS 20C. The earlier testing in which a suspension was tested showed the D-value to be 40.3 seconds, this study showed that longer D-values are obtained with STERIS 20C in suspension tests compared to dried film tests; however, the product has sufficient potency to achieve sterilization (12D) in times less than that recommended for use of the product.

In D-value testing (Gibraltar Report G-79769, see Appendix) of M. bovis (suspension) the D-value was 96.3 seconds, a time substantially longer than the D-values for the bacterial spores. Because this was only a single test, a plan was made with the contract laboratory (Gibraltar) to repeat testing with a minimum of three replicates to confirm or not this unexpected high value. However, due to the unexpected low recovery of organisms in the working inoculum by the test laboratory and the very long times to grow out the cultures (approximately 90 days) this task was put on hold and not completed. Testing with Mycobacterium inoculated bronchoscopes was, however, carried out (See Device Testing).

For an additional discussion of the sporicidal efficacy in shelf life as evaluated by D-values of B. subtilis see Shelf Life/Packaging Section. Table 3 summarizes the regulatory sporicidal required testing and other sporicidal testing carried out.

Table 3 Summary of Microbiological Testing

Test	Organisms	Carriers	Sterilant Conditions	Exposure Time	Temp.	Water	Test Site
MEC determination	*B. subtilis*	Suspension	Varying concentration	Variable	20° C	1000 ppm hard water, 5% serum	STERIS
AOAC Sporicidal	*B. subtilis,* *C. sporogenes*	360 penicylinders 360 suture loops	MEC, after reuse	20 min	20° C	1000 ppm hard water, 5% serum	STERIS
AOAC Sporicidal (confirmatory)	*B. subtilis* *C. sporogenes*	60 penicylinders 60 suture loops	MEC, after reuse	20 min	20° C	1000 ppm hard water, 5% serum	Gibraltar
AOAC Bactericidal	*S. choleraseuis* *Staph. aureus* *P. aeruginosa*	180 penicylinders 180 penicylinders 180 penicylinders	MEC, after reuse	5 min	20° C	1000 ppm hard water, 5% serum	Gibraltar
AOAC Fungicidal	*T. mentogrophytes*	10 replicates in suspensions	MEC, after reuse	5 min	20° C	1000 ppm hard water, 5% serum	Gibraltar
AOAC Tuberculocidal	*Mycobacterium bovis*	8 replicates in suspension	MEC, after reuse	10 and 20 min	20° C	1000 ppm hard water, 5% serum	Gibraltar
AOAC Virucidal	*Herpes* type 2 Poliovirus 2	8 replicates, suspension 16 replicates, suspension	MEC, after reuse	5, 10 and 20 min	20° C	1000 ppm hard water, 5% serum	Gibraltar
AOAC Sporicidal	*B. subtilis* *C. sporogenes*	360 penicylinders 360 suture loops	Use dilution, after reuse	20 min	20° C	Tap	STERIS
AOAC Sporicidal	*B. subtilis* *C. sporogenes*	360 penicylinders 360 suture loops	Use dilution, after reuse	20 min	25° C	Tap	STERIS
AOAC Bactericidal (confirmatory)	*Staph. aureus* *P. aeruginosa*	80 penicylinders 80 penicylinders	MEC, after reuse	5 and 10 min	20° C	1000 ppm hard water, 5% serum	STERIS
AOAC Fungicidal	*T. mentogrophytes*	80 replicates in suspension	MEC, after reuse	5 and 10 min	20° C	1000 ppm hard water, 5% serum	STERIS

OTHER MICROORGANISMS STUDIED

In addition to the FDA regulatory requirements to evaluate sterilants the sporicidal efficacy of sterilants study reports must be submitted indicating that the germicide passes the AOAC Tuberculocidal Test, AOAC Fungicidal Test, AOAC Use-Dilution Test for Staphylococcus aureus, Salmonella choleraesuis, Pseudomonas aeruginosa and tests on small/medium lipid/nonlipid viruses including Polio Type II and Herpes simplex. See Table 3 for a summary of this testing.

At MEC a total of 540 carriers (180 S. choleraesuis, 180 S. aureus, 180 P. aeruginosa) were tested with each of 3 different sterilant lots against S. choleraesuis ATCC 10708, Staphylococcus aureus ATCC 6538, and Pseudomonas aeruginosa ATCC 15442 (Gibraltar Report G-80109, see Appendix). The sterilant lots were at or below MEC (800 $\pm$ 50 ppm) after 8 hours simulated reuse with an exposure time of 5 minutes. All carriers were sterile.

At MEC replicate samples of Trichophyton mentagrophtyes ATCC 9533 were tested as spore suspensions with two sterilant lots (Gibraltar Report G-80107, see Appendix). STERIS 20C after 8 hours simulated reuse testing at below MEC was fungicidal after a 5 minute contact time.

At MEC the effectiveness against Mycobacterium bovis ATCC 35743 was substantiated with data derived from an 8 hour simulated reuse sterilant solution according to the EPA Tuberculocidal test method for each of two lots. Contact times were 10 and 20 minutes for replicate samples in an 8 hour simulated reuse sterilant solution made in 1000 ppm AOAC hard water with 5% calf serum at MEC. STERIS 20C was germicidal against M. bovis (Gibraltar Report G-80107, see Appendix).

At MEC EPA type virucidal testing after 8 hours of simulated reuse was performed on the STERIS 20C test solutions for each of two lots (Gibraltar Report G-79941, see Appendix). The viral titer was $\geq 10^{-5}$ $TCID_{50}$ /ml. The tissue culture medium used was RPMI 1640 with 5% serum or equivalent. In addition to the assay for effectiveness, each dilution 10^{-1} to 10^{-3} was tested for cytotoxicity. STERIS 20C tested in the presence of 1000 ppm AOAC hard water and 5% calf serum was completely virucidal after 8 hours of reuse against replicate samples of Herpes virus Type 2 after a contact time of 5 minutes at below MEC and of Poliovirus 2 at times of 10 and 20 minutes at or below MEC.

At MEC confirmatory sterility testing of bactericidal and fungicidal efficacy of STERIS 20C with 3 sterilant lots and using 80 inoculated stainless steel carriers per time point of P. aeruginosa ATCC 15442 and S. auerus ATCC 6538 (STERIS Technical Report T 94-016, see Appendix) exposed for 5 and 10 minutes and replicate samples of T. mentagrophytes ATCC 9533 spore suspensions exposed for 5 and 10 minutes. Sterility was achieved at both exposure times.

PHYSICAL AND CHEMICAL CHARACTERISTICS OF STERIS 20C

The following information addresses the data requirements of the U.S. Environmental Protection Agency: Pesticide Assessment Guidelines; Series 63, Physical and Chemical Characteristics, for STERIS 20C Sterilant.

Test substances include the technical grade of the active ingredients (TGAI): sodium perborate monohydrate, acetylsalicylic acid and tetracetylethylenediamine (TAED) in granulated form, and the blended use product, STERIS 20C Sterilant, which includes the TGAIs and the ingredients of the formula.

A number of sources for the same quality and technical grade of the active ingredients (perborate, acetylsalicylic acid, and TAED) have been identified. Referencing of the physical and chemical properties is made from a number of sources when reported values are given.

- Color

The color of the TGAIs and the blending end-use product is white to cream by visual inspection.

No coloring or discoloring agent(s) have been added.

- Physical State

The physical state of the TGAIs and the blended end-use product is a dry, crystalline, granular mixture at 20-25°C by visual inspection.

- Odor

Sodium perborate is ordorless.

Acetylsalicylic acid has a faint acid odor.

Tetracetylethylenediamine is odorless.

The blended end-use product has a light odor characteristic of acetylsalicylic acid.

- Melting Point

Sodium perborate monohydrate decomposes at 65°C.[4,5]

The melting point of acetylsalicylic acid is 133-135°C as determined by rapid heating.[6]

The melting point of TAED is granulated form is 149°C.[7]

Determination of melting point for the end-use product is not required under 40 CFR 158.120, Section 63-6.

- Boiling Point

The TGAIs exist as solids; therefore, determination of the boiling point for the TGAIs is not required under 40 CFR 158.120, Section 63-6.

Determination of boiling point for the end-use product is not required under 40 CFR 1580.120, Section 63-6.

- Density, Bulk Density, or Specific Gravity.

Sodium perborate has a bulk density of 0.5 - 0.6 g/ml.[5]

Acetylsalicylic acid has a density of 0.66 g/ml.[8]

TAED in granulated form has a density of 0.43-0.53 g/ml.[9]

The density of the blended end-use product is 0.69 g/ml.[10]

- Solubility

Acetylsalicylic acid has a water solubility of 1%.[6]

Sodium perborate monohydrate has a water solubility of 15 g/L at 20°C.[4]

TAED in granulated form has a water solubility of 0.2 g/L at 20°C.[11]

Determination of solubility for the end-use product is not required under 40 CFR 158.120, Section 63-8.

- Vapor Pressure

Determination of vapor pressure for the end-use product is not required under 40 CFR 158/120, Section 63-9 since the boiling point of the pure form of the TGAI's is below 30°C.

- Dissociation Constant

Acetylsalicylic acid has a dissociation constant (k) of 3.27×10^{-4} at 25°C.[8]

Sodium perborate monohydrate is an inorganic salt and is therefore 100% dissociated.[12]

TAED contains no ionic bonds; therefore, it has no dissociation constants.[12]

Determination of the dissociation constant for the end-use product is not required under 40 CFR 158.120, Section 63-10.

- Octanol/Water Partition Coefficient

Acetylsalicylic acid is not a non-polar organic therefore determination of an octanol/water partition coefficient is not required under 40 CFR 158.20, Section 63-11.

Sodium perborate is not a non-polar organic therefore determination of an octanol/water partition coefficient is not required under 40 EFR 158.120, Section 63-11.

TAED has a coefficient of log P_{ow} = -1.8 (calculated)[12] and 0.6449 (per HPLC method).[9]

Determination of an octanol/water partition coefficient for the end-use product is not required under 40 CFR 158.120, Section 63-11.

- pH

Sodium perborate monohydrate has a pH of 10.1-10.4 in a water solution of 15 g/L at 20°C[4] and a pH of 10.2 in a 1% solution.[5]

Acetylsalicylic acid has a pH of 4-5 in a 1% solution (saturated).[8]

TAED in granulated form pH = 7.50 in a 1% solution in distilled water at 20°C.

The mean pH of the water solubilized end-use product is 8.0 $\pm$ 0.2 per STERIS laboratory studies.[10]

- Stability

Sodium perborate thermal decomposition will occur above 65°C.[4]

Acetylsalicylic acid is stable for 3 years.[13]

TAED in granulated form is stable for at least 2 years.[12]

Stability of the end-use product is not required under 40 CFR 158.120, Section 63-13.

Product packaging contributes to stability of the product by (i) preventing exposure of container contents to moisture and/or light and (ii) maintains physical separation of the active and nonactive ingredients during shipping/storage/handling.

- Oxidizing/Reducing

  Determination of the oxidizing/reducing properties of the TGAIs is not required under 40 CFR 158.120, Section 63-14.

  The oxidizing/reducing properties of the end-use product have been studied (Monarch Analytical Laboratories Report COM 94-O-2538, see Appendix).

  Aliquots of the end-use product were exposed to either zinc metal (reducing agent), water, ammonium phosphate monobasic (fire extinguishing agent) or 1% aqueous potassium permanganate (oxidizing agent) at 23-25°C. No significant temperature change (less than 5°C) was recorded for the zinc, water and ammonium phosphate monobasic mixtures during the 24 hour cycle. For the water mixture some foaming was noted and a bleach-like odor detected. A 55°C temperature rise was noted for the mixture of 20C sterilant powders and potassium permanganate. Significant foaming occurred and a strong bleach like odor detected. The permanganate was decolorized.

  The addition of these agents to the end-use product is an exothermic reaction.

  An increase in temperature with mixing of the end-use product with aqueous solutions is in part related to the hydration of the end-use product. The additional rise in temperature above that of the addition of water alone, as in the case of potassium permanganate, is related to the oxidizing property of the end-use product.

- Flammability

  Determination of flammability characteristics of the TGAIs is not required under 40 CFR 158.120, Section 63-15.

  Determination of the flashpoint for the end-use product is not required under 40 CFR 158.120, Section 63-15 since the end-use product is not a liquid.

  Determination of flame extension characteristics are not required under 40 CFR 158.120, Section 63-15 since the end-use product is not an aerosol.

- Explodability

  Determination of the explodability of the TGAIs is not required under 40 CFR 158.120, Section 63-16.

The end-use product contains no known explosive ingredients therefore determination of impact explosion characteristics is not required under 40 CFR 158.120, Section 63-16.

Sodium perborate decomposes with liberation of heat and oxygen which may intensify fire in combustible surroundings. It is non-flammable and non-combustible.[4,5]

Acetylsalicylic acid dust accumulation on surfaces will burn rapidly when ignited. Dust dispersed in the air is a definite explosion hazard.[6,14]

TAED in granulated form will burn in air to give acetic acid and cyclic amine tars initially. Further heating will give carbon dioxide and NO_x.[6]

- Storage Stability

  STERIS has not entered into commercial manufacture of the product (STERIS 20C) therefore storage stability data on production blends packaged in the commercial package is not yet available. Stability studies of pre-production blends are in progress and reported separately.

- Viscosity

  Determination of viscosity for the TGAIs and/or the end-use product is not required under 40 CFR 158.120, Section 63-18 since the product is not a liquid.

- Miscibility

  Determination of miscibility for the TGAIs and/or the end-use product is not required under 40 CFR 158.120, Section 63-19 since the product is not an emulsifiable liquid and which does not bear directions for dilution with petroleum solvents.

- Corrosion Characteristics

  Determination of corrosion characteristics for the TGAIs is not required under 40 CFR 158.120, Section 63-20.

  Studies of the corrosive effects of the blended end-use product on the package are in progress and reported separately. No corrosion of the plastic package has been observed to date.

- Dielectric Breakdown Voltage

  Determination of dielectric breakdown voltage for the TGAIs is not required under 40 CFR 158.120, Section 63.21.

Determination of dielectric breakdown voltages for the end-use product is not required under 40 CFR 1580.120, Section 63-21 since the end-use product is not a non-conductant end-use liquid intended for use in or around electrical equipment.

SHELF LIFE/PACKAGING

In preparation of shelf life studies, package materials selection, package design, package integrity, manufacturing considerations and package opening requirements were reviewed. Polyethylene and polyethylene-nylon packaging films were selected as packaging materials based on considerations of cost, manufacturability, and performance requirements of a flexible pouch. The polyethylene-nylon materials, based on preliminary storage studies at elevated temperatures up to 45°C, were selected for the shelf life studies on the sterilant.

Upon completion of the early feasibility testing of the sterilant, three lots (designated 1, 2, and 3) of builders (all components of the sterilant except the acetyl donors) were produced in a kitchen blending mixer. Based upon quality assurance testing these lots were accepted for further testing (STERIS Report T94-002 and Addendum, see Appendix). For one of these lots tested at 12 months the results were also acceptable.

To more closely approximate manufacturing conditions two pre-commercial lots (designated by a P) of STERIS 20C builders were produced in a production VEE-blender. The baseline results of the dissolution assay showed a slightly shorter time (33-34 minutes) for lots P-1 and P-2 than for lots 1, 2 and 3. As seen with lots 1, 2, and 3 of STERIS 20C, all of the components dissolved within 5 minutes with the exception of the encapsulated TAED. The blended P-1 and P-2 builder lots showed less variability than the lots 1, 2, and 3 produced in the kitchen mixer. These lots and P-1 and P-2 were accepted for testing based on the Quality Assurance assay results (STERIS Report T-94-007, see Appendix) and were used for shelf life/stability studies in real and accelerated times. For shelf life assessments quantities of each of these lots were packaged in the polyethylene-nylon packets, one containing the builders components and the other containing the activators.

For determination of shelf life time, the time between sterilant production and packaging was considered negligible. Ten sterilant packets from lots P-1 and P-2 for each time point to be studied were placed upright in a cardboard box, and stored in the laboratory and ambient relative humidity (50 ± 5%). In addition, ten sterilant packets each were placed upright in a cardboard box and stored in an incubator at 45 ± 1.0°C and ambient relative humidity. All packaging/storage conditions were defined to simulate actual production criteria.

For the shelf life study at the accelerated temperature, real time equivalents were calculated according to the $Q_{10} = 2$ concept. This method for shelf life determination is based on the concept that a 10°C increase in temperature will double the rate of a chemical reaction.[15] The Q_{10} value represents the ratio of the rates of two reactions which are ten degrees apart. If the reaction rate is doubled, then $Q_{10} = 2$. Table 4 outlines the shelf life time equivalents for pre-commercial STERIS 20C lots.

Table 4 Calculation of Shelf Life Time Equivalents for Pre-Commercial STERIS 20C Lots P-1 and P-2

(test temperature °C) - (ambient temperature difference °C) = X

Where X is the temperature difference in °C

$$2^{X/10} = Y$$

Where Y is the thermodynamic temperature coefficient (acceleration factor)

$$365/Y = Z$$

Where Z is the number of days at elevated temperature which are equivalent to one year

For an ambient laboratory temperature of 22.8°C, the following time equivalents were calculated for the given temperatures

For a storage temperature of 45°C:

X = 22.2°C
Y = 4.66

Therefore:

3 months = 20 days
6 months = 39 days
9 months = 59 days
12 months = 78 days
15 months = 98 days
18 months = 118 days
24 months = 157 days

Shelf life stability testing was performed to assess the potency stability resulting from packaging and storage of the sterilant in order to support the expiration data of the product. The STERIS 20C sterilant was evaluated on a physical, chemical and microbiological basis. STERIS Report T 94-013 (see Appendix) outlines the results of testing through two years accelerated time and nine months real time (physical and chemical testing only). Testing through 36 months was carried out (Table 5). Through these times there were no significant change from baseline values in the physical, chemical and microbiological efficacy due to degradation or environmental impact from storage conditions or the container. No discoloration or loss of vacuum, and no change in material flexibility or tear resistance of the package was noted. No clumping or visual degradation of the powdered sterilant for any of the shelf life samples tested was noted. Peracetic acid generation as monitored from 30 minutes to 10 hours gave a mean PAA concentration of greater than 900 ppm over eight hours. All values of percent active oxygen of sodium perborate were higher than a minimal specification of 5.1%. All values for salicylic acid were below the incoming raw material QA specification (<0.5% salicylic acid) for acetylsalicylic acid. Baseline values for % TAED based on the Certificate of Analysis provided by the vendor were all within the acceptable range because PAA generation for each sample was above the minimum effective concentration (MEC) for the recommended sterilant reuse life of eight hours. Microbiological efficacy testing of the accelerated shelf life samples showed a minor but non-significant increase in D-values over the 24 months of study (STERIS Technical Report T 94-014.1, see Appendix) demonstrating the preservation of microbiological effectiveness. The 12D values were within the recommended twenty minute sterilization time.

In testing of the sterilant's efficacy as evaluated by D-values for Bacillus subtilis (Table 6) beginning at 9 months after baseline the D-values were significantly longer than those at baseline and the values at 12 and 24 months equivalent times for the accelerated shelf life samples. This was despite the fact that the chemical evaluation of the active components of the sterilant showed less degradation in comparison to the accelerated shelf life samples previously evaluated at 12 months equivalent time and that mean peracetic acid concentration over an 8 hour period and all time points between were appreciably above MEC. The mean 12D values at 12 months real time storage were still less than one half the 20 minute recommended device contact time.

At 12 months real time the active oxygen values were higher than the minimal lot specification and the percent salicylic acid (hydrolysis product of acetylsalicylic acid) was below the maximum allowed specification and showed less degradation of these active components in comparison to the 12 month accelerated samples. Peracetic acid generation and time stability evaluations indicated that these 12 month real time samples performed as well as the baseline and the 12 month accelerated time samples. The 12 month real time mean D-values for B. subtilis for the two pre-commercial lots were 47.8 and 32.6 seconds versus 10.6 and 18.2 seconds at baseline and 9.8 and 11.3 seconds at 12 month accelerated time and 12.1 and 19.2 seconds at 24 month accelerated time. These increased D-value times still provide 12 D times of less than one half the 20 minutes recommended device soak times; however, these values appear inconsistent with prior testing and the good results of the shelf life chemistry studies.

Table 5. Accelerated Shelf Life Testing

Assay	Baseline		3 month		6 month		9 month		12 month	
	P-1	P-2	P-1	P-2	P-1	P-2	P-1	P-2	P-1	P-2
% active Oxygen Sodium Perborate Lot # 3/1574	6.35±0.07	6.35±0.07	6.35±0.12	6.45±0.13	NA	NA	6.03±0.06	6.07±0.07	5.68±0.19	5.41±0.12
% Salicylic Acid ASA lot 9309680054	0.115±0.005	0.115±0.005	0.113±0.003	0.113±0.003	0.113±0.003	0.113±0.003	0.112±0.007	0.117±0.007	0.127±0.007	0.127±0.007
%TAED Lot # 33120662	84.7	84.7	81.1	81.1	NA	NA	82.1	82.1	NA	NA
pH (mean 30min-8.5 hrs)	7.6±1.5	7.6±1.5	7.5±1.5	7.5±1.5	7.6±1.5	7.7±1.5	7.6±1.5	7.6±1.5	7.7±1.5	7.8±1.5
PAA Generation at (20°C) peak conc. (ppm) (30 min- 8.5 hour)	1236±31	1210±116	1204±19	1251±44	1247±66	1267±50	1292±68	1248±10	1351±51	1418±42
low conc. (ppm) (30 min- 8.5 hour)	985±87	823±131	1045±54	819±20	997±54	928±91	893±34	907±86	996±16	946±8
mean conc.(ppm) (30 min- 8.5 hour)	1085±42	1034±27	1171±51	1145±30	1175±44	1165±31	1132±21	1133±30	1205±43	1210±35
Conc.(ppm) @ 30 min.	1016±117	823±131	1076±130	819±20	997±54	1137±10	893±34	907±86	1254±126	1419±42
Conc. (ppm) @ 8.5 hour	1011±62	957±76	1045±54	1080±48	1014±55	1082±105	1002±22	1029±26	996±16	946±8
D-value (Mean)										
D (sec.)	10.56±4.57	18.16±8.78	NA	NA	NA	NA	NA	NA	9.77±1.93	11.26±0.85
12D (sec.)	126.7±54.9	218.04±105.4							117.2±23.2	135.1±10.2
End Point (sec)	<180	<240							<180	>120

NA - data is unavailable.

Table 5. Continued. Accelerated Shelf Life Testing

Assay	Baseline		15 month		18 month		24 month		30 month		36 month	
	P-1	P-2	P-1	P-2	P-1	P-2	P-1	P-2	P-1	P-2	P-1	P-2
% active Oxygen Sodium Perborate Lot # 3/1574	6.35±0.07	6.35±0.07	5.57±0.15	5.93±0.32	5.47±0.15	5.78±0.07	5.53±0.14	5.48±0.14	NA	NA	NA	NA
% Salicylic Acid ASA lot 9309680054	0.115±0.005	0.115±0.005	0.121±0.007	0.121±0.007	0.114±0.010	0.114±0.010	0.117±0.009	0.117±0.01	NA	NA	NA	NA
% TAED as MYKON Lot # 33120662	84.7	84.7	81	81	NA	NA	80.8	80.8	NA	NA	NA	NA
pH (mean 30min - 8.5 hrs)	7.6±1.5	7.6±1.5	7.6±1.5	7.4±1.4	7.6±1.5	7.5±1.5	7.6±1.5	7.8±1.5	7.3±1.0	7.3±1.0	7.7±1.5	7.5±1.5
PAA Generation: at 20°C peak conc. (ppm) (30 min- 8.5 hour)	1236±31	1210±116	1315±13	1302±29	1270±54	1098±116	1312±15	1353±76	1401±25	1394±38	1436±84	1282±74
low conc. (ppm) (30 min- 8.5 hour)	985±87	823±131	1036±28	840±45	793±95	659±85	956±152	989±75	746±24	707±52	1026±96	877±72
mean conc.(ppm) (30 min- 8.5 hour)	1085±42	1034±27	1173±66	1152±41	981±90	946±102	1060±67	1177±68	1295±43	1281±74	1262±53	1193±110
Conc.(ppm) @ 30 min.	1016±117	823±131	1029±255	840±45	793±95	659±85	956±152	1191±141	746±42	707±70	1109±116	877±202
Conc. (ppm) @ 8.5 hour	1011±62	957±76	1036±4	1110±31	923±39	893±39	1109±88	989±76	1265±88	1268±45	1105±29	1164±77
D-value (Mean)												
D (sec.)	10.56±4.57	18.17±8.78	NA	NA	NA	NA	12.13±3.99	19.16±4.80	77.9±47.1	68.9±38.3	52.89±24.17	NA
12D (sec.)	126.7±54.9	218.04±105.4					145.56±47.9	229.9±57.6	934.8	826.8	634.68	
end point (sec)	<180	<240					<240	<300	>720	>720	>720	

% TAED was analyzed by Vendor
NA - data is not available

Table 6. Real Time Shelf Life Testing

Assay	Baseline		3 month		6 month		9 month		12 month	
	P-1	P-2	P-1	P-2	P-1	P-2	P-1	P-2	P-1	P-2
% active Oxygen Sodium Perborate Lot # 3/1574	6.35±0.07	6.35±0.07	6.52 ±0.17	6.52±0.27	6.11±0.14	6.11±0.14	6.31±0.04	6.21±0.06	6.26±0.007	6.28±0.002
% Salicylic Acid ASA lot 9309680054	0.115±0.005	0.115±0.005	0.108±0.005	0.108±0.0047	0.101±0.081	0.101±0.081	0.110±0.001	0.110±0.001	0.105±0.001	0.105±0.001
% TAED as MYKON Lot # 33120662	84.7	84.7	80.3	80.3	NA	NA	NA	NA	NA	NA
pH (mean 30min - 8.5 hrs)	7.7±1.0	7.7±1.0	7.7±1.5	7.7±1.5	7.6±1.5	7.5±1.5	7.5±1.5	7.5±1.5	7.5±0.0	7.6±0.0
PAA Generation at 20°C peak conc. (ppm) (30 min- 8.5 hour)	1236±31	1210±116	1231±21	1236±41	1253±45	1244±81	1150±20	1179±41	1530±12	1562±14
low conc. (ppm) (30 min- 8.5 hour)	985±87	823±131	937±35	899±40	865±68	974±55	855.3±9	772.3±71	1008±17	1028±35
mean conc.(ppm) (30 min- 8.5 hour)	1085±42	1034±27	1117±35	1118±69	1095±42	1070±42	1080±24.7	1058±32	1264±15	1399±25
Conc.(ppm) @ 30 min.	1016±117	823±131	987±69	1002±175	865±68	974±55	855.3±9	772.3±71	1174±5	1362±42
Conc. (ppm) @ 8.5 hour	1011±62	957±76	1040±16	955±43	1067±35	1093±64	1104.7±17	1106.3±24	1008±17	1028±35
D-value (Mean) D (sec.) 12D (sec.) end point (sec)	10.56±4.57 126.7±54.8 <180	18.16±8.78 218.04±105.4 <240	NA	NA	NA	NA	41.20±6.26 494.4±160.3 >360	42.36±10.74 508.4±128.9 >360	47.75±8.17 572.99±98.04 <480	32.60±21.17 391.22±253.99 <480

NA - data is unavailable.

Table 6. Continued. Real Time Shelf Life Testing

Assay	Baseline		15 month		18 month		24 month	
	P-1	P-2	P-1	P-2	P-1	P-2	P-1	P-2
% active Oxygen Sodium Perborate Lot # 3/1574	6.35±0.07	6.35±0.07	6.86±0.47	5.90±0.29	6.26±0.01	6.28±0.02	5.98±0.03	6.04±0.05
% Salicylic Acid ASA lot 9309680054	0.115±0.005	0.115±0.005	0.104±0.004	0.104±0.004	0.105±0.001	0.105±0.001	0.135±0.001	0.135±0.001
% TAED as MYKON Lot # 33120662	84.7	84.7	NA	NA	80.8	80.8	NA	NA
pH (mean 30min - 8.5 hrs)	7.7±1.0	7.7±1.0	7.6±1.0	7.6±1.5	7.5±1.0	7.5±1.0	7.51±1.0	7.50±1.0
PAA Generation at 20°C peak conc. (ppm) (30 min- 8.5 hour)	1236±31	1210±116	1407±29	1401±24	1176±25	1222±19	1405±64	1402±18
low conc. (ppm) (30 min- 8.5 hour)	985±87	823±131	1090±73	1055±54	664±26	609±21	1078±60	999±67
±mean conc.(ppm) (30 min- 8.5 hour)	1085±42	1034±27	1249±137	1252±136	915±122	917±102	1262±112	1232±107
Conc.(ppm) @ 30 min.	1016±117	823±131	1184±120	1339±114	1039±117	968±43	1100±117	1118±30
±Conc. (ppm) @ 8.5 hour	1011±62	957±76	1090±73	1055±54	664±26	609±21	1078±60	1093±13
D-value (Mean) D (sec.) 12D (sec.) end point (sec)	10.56±4.57 126.7±54.8 <180	18.16±8.78 218.04±105.4 <240	37.18±9.63* 446.2±115.6* >600	54.81±6.1* 657.72±7.32* >600	58.46±7.39 701.5±88.68 >720	59.65±8.84 715.8±106.08 >720	259.8±65.8 3117.6 >780	311.4 3736.8 >780

NA - data is unavailable.

* Completed using 15 month non packaged material

As shown in Table 6 the minor changes in the chemistries do not correlate with the very significant increases in the D-values for B. subtilis, in particular after 12 months of real time storage. As an independent assessment of the B. subtilis D-value Gibraltar Laboratories determined that the D-value for lot P-2, stored for 24 months real time, had a value of 103 seconds . While this was appreciably lower than that determined by STERIS, it is still significantly higher than that of this lot at baseline and through 18 months of storage.

In AOAC carrier studies following our simulated reuse protocol with lot P-2 of 25 months of age, a number of positives (i.e. sterilization failures) were noted, in particular for the suture loops. In additional testing of carriers at 30 minute time exposure at or above 800 ppm peracetic acid, and also at two times its dose, positives were noted as well.

Because of the longer D-values of B. subtilis seen in real time in shelf life of 9 months and greater and the inability to sterilize consistently some rigid devices and flexible lumened scopes new lots of the 20C were produced. Expecting that the increased D-values and failures of carriers were related to storage of the product, three new lots of the sterilant, lots P-5, P-6 and P-7, were produced in manufacturing equipment and evaluated at baseline. Table 7 outlines the results at baseline for these new lots in comparison to lots P-1 and P-2. In general, the chemical results of the new lots indicated that they performed at least as well as the older lots in terms of peracetic acid generation. However, the D-values for these lots were extremely long and 12D times were appreciably longer than the 20 minute contact time proposed for this product. Carrier studies resulted in a number of positives. In simulated use testing with one of these new lots, positives were noted on nonlumened rigid devices tested.

Also as a result of these concerns STERIS requested and held a meeting on March 20, 1996 with the Army at Ft. Detrick to discuss: (1) technical issues, (2) contractual issues, and (3) next steps that STERIS should take that would be agreeable to the Army.

Table 7 Summary Of Baseline Testing For STERIS 20C Lots P-1, P-2, P-5, P-6, P-7

Assay	P-1	P-2	P-5	P-6	P-7
Pour Density (g/ml)	0.68± 0.01	0.69± 0.01	0.67± 0.004	0.67± 0.004	0.70± 0.002
Benzotriazoles (%)	2.23 ± 0.14	2.20 ± 0.15	2.39 ± 0.03	2.39 ± 0.03	2.42 ± 0.03
Dissolution Time (min)	5.0 ± 0.5	4.5 ± 0.5	3.5 ± 0.5	3.5 ± 0.5	3.3 ± 0.5
pH of Builders	8.20 ± 0.10	8.10 ± 0.10	8.20 ± 0.02	8.35 ± 0.02	8.24 ± 0.04
Active Oxygen (%)	6.35 ± 0.07	6.49 ± 0.10	7.09 ± 0.02	7.09 ± 0.02	7.10 ± 0.01
Salicylic Acid (%)	0.115±0.004	0.115±0.004	0.015±0.001	0.015±0.001	0.015±0.001
Mean [PAA] 0.5-8.5 hrs. (ppm)	1085 ± 42	1034 ± 27	1254 ± 57	1322 ± 50	1296 ± 57
Peak [PAA]	1236 ± 31	1210 ± 116	1545 ± 80	1481 ± 53	1539 ± 74
Low [PAA]	985 ± 87	823 ± 131	992 ± 56	1116 ± 44	1053 ± 11
[PAA] at 0.5 hrs.	1016 ± 117	823 ± 131	1309 ± 31	1157 ± 88	1316 ± 122
[PAA] at 8.5 hrs.	1011 ± 62	957 ± 76	992 ± 56	1119 ± 27	1054 ± 4
Mean pH (0.5-8.5 hrs.)	7.60 ± 1.5	7.60 ± 1.5	7.73 ± 0.03	7.61 ± 0.03	7.63 ± 0.03
D-value (sec.) **12D (min.)** **endpoint (min.)**	10.56± 4.57 2.1 <3	18.16± 8.78 3.6 <4	164±59.8 32.8 9	253.3±1.6 50.6 12	154.3±120.5 30.9 10

Note: The D-values for lots P-1 and P-2 were performed using the quantal method at PAA concentrations of 1000 - 1100 ppm at 19.5 - 20.0° C in 1000 ppm of AOAC Hardwater and 5% Serum. The D-values for lot P-5, P-6, and P-7 were performed using the direct plate method at use dilution of PAA at 20.0° C in deionized water.

TOXICOLOGY

The sterilant has been formulated with chemical agents that have been used in other STERIS peracetic acid based sterilants with the exception of one component, TAED. The individual chemistries used are common to many consumer products such as dental cleansers, detergents, and over the counter drugs. A review of the toxicological data of the acetyl donor tetraacetyl ethylenediamine (TAED) indicated it to be relatively nontoxic. Per the EPA end user exposure must be evaluated and per the FDA toxicological evaluation of any residue remaining on a reusable medical devices after they have been treated with the germicide and after the residue reduction step.

Various toxicological tests of the use dilution were conducted by independent laboratories including: acute dermal, primary dermal irritation, ocular irritation, acute oral, Beuhler dermal hypersensitivity, and fish toxicity. A summary of the results is given below.

Acute dermal test - Under the conditions of this test (Gibraltar Report G-76647, see Appendix) STERIS 20C is considered to be non-toxic with an LD_{50} of greater than 2 grams per kilogram in rabbits. All rabbits survived in a limit test of 2 grams per kilogram of body weight. The use dilution did not produce erythema or edema at 24 or 72 hours. All animals appeared healthy throughout the test period and no product of abnormalities were observed at the terminal necropsy.

Primary dermal irritation - Under the condition of the primary dermal irritation test (Gibraltar Report G-76364, see Appendix), the use dilution was not a primary dermal irritant and was noted as non-irritating.

Ocular irritation - Under the test conditions of the ocular irritation test (Gibraltar Report G-76468, see Appendix), the use dilution was not considered an irritant to the eyes of rabbits. There were no signs of corneal opacity, iritis or conjunctivitis. All rabbits were healthy throughout the 3 day test period.

Acute oral - Under the conditions of the acute oral toxicity test (Gibraltar Report G-76487, see Appendix), the oral LD_{50} of the use dilution in rats was greater than 5 grams per kilogram and non-toxic. All rats survived 14 days and remained healthy throughout the test period. No abnormalities were observed at terminal necropsy.

Beuhler dermal hypersensitivity - The Beuhler hypersensitivity test (Gibraltar Report G-77120, see Appendix) on the use dilution tested at full strength did not produce an allergic contact dermatitis reaction in guinea pigs. The product is non-sensitizing and non-irritating.

Fish toxicity - The acute toxicity of the formulation to fathead minnows were assessed using the nominal sterilant concentration of 1000 mg/L with concurrent dilution water controls (Analytical Bio-Chemistry Laboratories (ABC) Report #41688, see Appendix). The LC_{50} was greater than 1000 mg/L. The 96-hour no-observed effect concentration (NOEC) was 1000 mg/L which was

based on the lack of mortality and abnormal effects at this concentration. The above testing has shown that the formulation is safe.

Testing was conducted (STERIS Report T 94-021, see Appendix) on representative medical devices initially and after single and repetitive exposure to standard manual soak 20C sterilant sterilization cycles (soak time of 20 minutes at 20°C). This testing is designed to assess the rinse methodology and to validate if any significant levels of residues were recoverable following the sterile water rinses or if residues accumulate on devices following repeated process exposures. The devices tested were the Pentax bronchoscope model FB-18X, the Karl Storz arthoscope model 7200° Hopkins, V. Mueller 7 inch scissor model SU 1992, a Thompson dental pick model Tactiletone Probe 4-23 Explorer, and a Bard dilator model 000275.

Following single and multiple sterilant process cycles no significant differences in extractable sterilant residues were noted on the devices. The employment of the three sterile water rinses of one minute each is sufficient to reduce 20C sterilant residues on the devices tested.

MATERIALS COMPATIBILITY

Materials evaluations were carried out to determine the effect of the sterilant on materials and to make recommendations for its use. Materials testing was conducted in vitro with static continuous long term contact, up to 2000 equivalent cycles, with STERIS 20C use dilution sterilant. Such testing is complimentary to device testing and permits accelerated evaluation of the materials in comparison to simulated use testing. Materials representative of the type used in the construction of medical devices intended to be in contact with the sterilant at 20°C were evaluated (STERIS Technical Reports T 94-012, T 94-024, and T 94-044, see Appendix; due to the lengths of these reports their individual appendixes are not included). Comparative statistical analyses were also made with the materials prior to contact with STERIS 20C and with an aqueous environment (tap water). Materials samples were immersed in glass beakers and immersed in STERIS 20C use dilution at 20°C made with tap water and maintained at 20°C. Tactile properties and color changes were measured and recorded on a regular basis at the time of sterilant change. Physical properties (dimensions, weight and hardness for plastics only) were measured and recorded at 0, 1000, and 2000 equivalent cycles or at other times if any significant physical changes were noted. Table 8 lists the materials studied.

Studies showed no significant changes from baseline with STERIS 20C exposure or if changes were noted they were not significantly different from water contact alone. The materials are compatible with STERIS 20C sterilant solution.

Table 8 Materials Evaluated With STERIS 20C

ADHESIVES
Araldite®

Armstrong C7:W

Epotech® 301-2

Tyrite®

ELASTOMERS
Ethylene propylene diene (EPDM)

RTV 133

Silicone rubber

Urethane
- ESTANE®
- PELLETHANE®

METALS
Aluminum 3003
-untreated

Aluminum 6061
- anodized
- untreated

Brass

Brass 360

Nickel-plated Cooper

Stainless steel 17-4PH

Stainless steel 303

Stainless steel 304

Stainless steel 316L

Stainless steel 410

PROCESSES
Anodization
-hard coat
- soft coat

Nickel-plating

PLASTICS/NON-METALS
Acrylonitrile butadiene styrene (ABS)

Acetal copolymer
- Delrin ® 500

Borosilicate glass
Ethylene propylene

Ethylene propylene diene (EPDM)

Polyamide
-Nylon 6/6

Polycarbonate (PC)
- Lexan® 9034
- Lexan® UHME

Polychloroprene
- Neoprene

Polyether imide (PEI)
-ULTEM® 1000

Polyetherether ketone (PEEK)

Polyethylene (PE)
- high density

Polyphenyl oxide/polystyrene
-Noryl® EN-185

Polypropylene

Polystyrene

Polytetrafluoroethylene (PTFE)
-expanded
-unexpanded

Polyvinyl chloride (PVC)
-unplasticized

Polyurethane

EFFECT OF PRECLEANER RESIDUALS

Because devices are precleaned prior to sterilization in clinical use and because improper rinsing may contribute to precleaner carry-over into the sterilant the effect of precleaners was assessed. Up to three percent of each of five representative precleaners, including three enzyme containing products, were tested for compatibility with STERIS 20C sterilant at 20°C, 40°C and 50° (STERIS Report T 94-018, see Appendix). The peracetic acid concentrations for the sterilant solutions containing three percent of the precleaners were comparable to those measured for the control which contained only tap water. These results indicate that if residues of these cleaning agents are carried over to the sterilant solution they are compatible with STERIS 20C.

MEDICAL DEVICE TESTING

The FDA Guidance Document (dated 4/26/95)[2] concerning the submissions for liquid chemical germicides specifies the need to perform device sterility and compatibility testing to validate a given sterilant. Medical/dental devices having representative materials and design features have been evaluated with STERIS 20C at 20°C at either MEC (800 $\pm$ 50 ppm peracetic acid) or at use dilution.

Prior to exposure to the sterilant the devices were evaluated to determine its material condition and functionality. The prior testing at STERIS of used devices was noted. The device sites and lumens (where applicable) for sterile challenge were chosen to be representative of areas of the devices where sterilant fluid contact would be most difficult to achieve or to be areas of clinical relevance as being patient contact sites. Devices tested had lumens, hinges and various etched surfaces as well as other features that may impede cleaning and penetration of the sterilant. Each device surface was swabbed with a $\geq 10^7$ spore suspension of B. subtilis. Lumens were inoculated with 5 ml of B. subtilis spore suspension (total inoculum of 10^7 cfu/lumen) injected with a syringe. All surface sites/lumens on devices were inoculated in each study regardless of whether the site was harvested. Inoculum was allowed to air dry for $\geq$ 30 minutes to simulate clinical conditions under which sterilization of devices could not be performed immediately after cleaning of the device.

The devices were placed in a soak pan filled with the sterilant of a sufficient volume so as to cover all device surfaces. Variable sterilant volumes were used due to the variability in the number (one to five) of devices processed together and the size and shape of these devices. Sterilant was made using AOAC hard water of $\geq$800 ppm as $CaCO_3$ with 5% bovine serum. Peracetic acid concentration of the sterilant was determined using a spectrophotometric assay. The temperature and pH of the solution were also recorded.

After soaking in the sterilant at 20°C the devices were rinsed in three sterile water rinse baths which were the same volume for a minimum time of one minute for each bath. Lumens were flushed with sterile water three times in each rinse bath.

Following completion of rinsing, the individual surface sites were harvested with swabs moistened in sterile tryptic soy broth (TSB). Each swab was aseptically transferred to TSB (growth medium) and incubated at 37°C. The lumens were harvested by injecting them with 20 ml deionized sterile water using a sterile syringe and collecting into 20 ml of double strength TSB. The media was incubated at 37°C. Cultures were read at 3 and 7 days for evidence of growth. Devices were cleaned/scrubbed using a detergent and rinsed with tap water between cycles to simulate treatment in clinical use.

To make a claim of sterility, five consecutive challenges at each site must be shown to be sterile with demonstrated positive growth in control testing and a recoverable bioburden of $\geq 10^3$ was chosen to represent a bioburden challenge greater than typically seen on used medical devices. A control cycle consists of all conditions being the same but the acetyl donors are omitted. Growth of the cultures from the positive control at 3 days is an indication that a sufficient number of spores remain on the device to constitute a valid challenge. Sites were also quantified with the use of tryptic soy agar pour plates.

Surface recoverable bioburden challenges were performed by swabbing each site with a spore suspension of $\geq 10^7$ cfu/ml. Sites were immediately harvested with swabs moistened in sterile TSB. Each swab was then aseptically transferred to a test tube containing 10 ml of TSB. Lumen recoverable bioburdens were performed by inoculating the lumens with a five ml spore suspension (total inoculum $\geq 10^7$ cfu). They were then harvested by injected 20 ml sterile deionized water through them with collection into 20 ml of a double concentration of TSB. Several dilutions were made from TSB and double concentration TSB tubes and cultures were plated into tryptic soy agar. Plates were incubated at 37°C and were read after two days incubation to determine colony forming units (CFU)/ml.

Recoverable bioburden was performed three separate times on each site for all devices. Recoverable bioburden measurements demonstrate the minimum quantity of microorganisms which can successfully be retrieved by the harvesting methods as it is recognized that subsequent swabbing or lumen perfusion may recover additional organism.

Any evidence of microbiological growth (test organism or contaminant) in the trials is considered nonsterile and the culturing sequence is repeated for that site. For this reason more than the minimum 6 cycles (one control and five standard trials) may be run per device.

Table 9 lists the devices successfully sterilized at the minimum effective concentration of 800 ± 50 ppm peracetic acid. This table also notes the STERIS report contained in the Appendix where the details of each device study can be found including the inoculation procedures and device sites studied. The sterilant age ranged from four to eighteen months for the device testing. Table 10 lists the device features of these devices. Five consecutive replicate samples for each device surface site and lumen were sterile. Control cycles (acetyl donors omitted) were positive for growth of the test organism and recoverable bioburdens were shown to be $\geq 10^3$ cfu for each

surface site/lumen tested. Each device met criteria for sterilization. No significant material property changes or device functionality changes were noted in short term testing.

Evaluation for compatibility and functionality of the medical devices for which simulated use testing with B. subtilis confirmed the ability of STERIS 20C to sterilize at 20°C for 20 minutes was completed through 100 cycles. All devices were shown to be compatible.

A number of representative devices as listed in Table 11 were subjected to standard cycles with STERIS 20C use dilution (STERIS Report T 94-011). These devices were successfully sterilized. The control cycles (acetyl donors omitted) were positive for growth of the test organism and recoverable bioburden was shown to be $\geq 10^3$ cfu for each surface site/lumen tested. Each device met criteria for sterilization (five consecutive cycles being sterile). Further, no significant material property changes or device functionality changes were noted.

Latex gloves were used in the processing of all devices for handling purposes. No difficulty was noted with prolonged exposure of the gloves to the sterilant. Further, in permeability testing of liquid STERIS 20C with glove samples (Maxxus latex, N-DEX nitrile 6 mil, Neutralon on brown latex, Fisher vinyl 5 mil, and Best neoprene) no measurable permeation through any of the samples took place within two hours of exposure (Akron Rubber Development Laboratory Report 32918).

Table 9 Devices Successfully Sterilized Under MEC Conditions At 20°C

	Report No.
Arthroscope, Karl Storz 7200A0°	T 95-026.2
Trocar, Karl Storz 27026BO	T 95-027.2
Microsurgical Tweezers, Pilling 42-5170, S/N 113	T 95-028.2
Microsurgical Scissors, Storz E3301	T 95-029.1
Ocular Lens, Ocular Instruments Inc.	T 95-030.1
Microsurgical Forceps, Pilling 42-7275, S/N 17B	T 95-031
Dental Pick, Thompson Tactile Tone SS Probe Explorer 4-23	T 95-032
Hemostat, Pakistan	T 95-033
Biopsy Forceps, Pentax KA2218CS, S/N A07671	T 95-034.1
Bougie, Medovation	T 95-035
Camera w/Coupler, Cabot Medical	T 95-036
Microsurgical Hemostat, V. Mueller CH8610	T 95-037
Scissors (Standard), Storz 095	T 95-038
Laserscope, CeramOptec	T 95-039
Angioscope, Applied Vascular ANG-080-RIOK	T 95-040
Vaginal Speculum, Med Peterson S2110	T 95-041
Cystoscope, Circon AUR-8	T 95-042
Dilator, Bard 000275	T 95-043

Table 10 Representative Device Materials/Feature of Devices Evaluated

	Devices																	
Features	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
Lumens										X					X		X	X
Fiberoptics	X										X			X	X		X	
Optical lens	X				X						X				X		X	
Moving parts			X	X		X		X	X		X	X	X			X	X	
Joint mechanism				X		X		X	X			X	X			X		
Cutting edges				X					X				X					
Multiple materials	X	X			X		X		X	X	X			X	X		X	
Plastics	X	X			X		X		X	X	X			X	X		X	X
Metals	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	
Adhesives	X				X		X		X		X			X	X		X	
Flexible									X	X	X			X	X		X	X
Covered Electrical connectors											X							

1 - Arthroscope
2 - Trocar
3 - Microsurgical Tweezers
4 - Microsurgical Scissors
5 - Ocular lens
6 - Microsurgical Forceps
7-Dental Pick
8 - Hemostat
9 - Biopsy Forceps
10 - Bougie
11 - Camera w/coupler
12 - Microsurgical Hemostat
13-Scissors (standard)
14 - Laserscope
15 - Angioscope
16 - Vaginal Speculum
17 - Cystoscope
18 - Dilator

Table 11 Representative Devices Tested With STERIS 20C At Use Dilution At 20°C

Biopsy Forceps, Pentax KA2218CS
Resectoscope Sheath with two stopcoaks, Karl Storz 27026B
Microsurgical Hemostat, V. Mueller CH8610
Dilator, Bard 000275
Dental Pick, Thompson Tactile Tone SS Probe Explorer 4-23

In addition to the testing conducted on the above devices there were devices which were not able to be consistently sterilized 20°C in 20 minute soaks. In particular the flexible lumened endoscopes, such as the bronchoscope, colonoscope and gastroscope, and other devices such as graspers, light cable, urethroscope, high speed dental handpieces, low speed dental handpieces and a mouthpiece.

For some devices such as the camera coupler, scissors and sheath inconsistent sterilization was seen with different models or the same model tested at different times. In testing of other lumened devices (cystoscope, angioscope, bougie, and dilator) sterilization validation was met. Thus, inconsistency of results and the inability a priori to determine which of these sites could be sterilized in our simulated use testing raised additional concerns (over and above the longer D-values seen in real time shelf studies) as to the practical efficacy of this product despite successful regulatory testing to show that the product is sporicidal, bactericidal, fungicidal, tuberculocidal and virucidal. In addition, in testing under the same conditions the surface sites of the bronchoscope met sterilization validation and surface sites on the gastroscope and colonoscope were shown to be sterile although growth was seen from the lumens. These results did not suggest the ineffectiveness of the sterilant solution as the cause of positive growth in the lumens but rather the inability to deliver the sterilant to device sites.

This inability to meet STERIS sterilization validation criteria, in particular for the lumened flexible scopes and the commercial importance that the product be able to be used with these devices required STERIS's continued evaluation and delayed the decision to proceed with in-use testing.

In an effort to develop a claim for the sterilant with flexible lumened devices STERIS commissioned an independent test lab to do testing with Mycobacterium. Given that sterilization could be accomplished with this organism a high level disinfection claim could be made. In testing carried out at Gibraltar Laboratories (Gibraltar Report G-87766, see Appendix) a bronchoscope was evaluated twice with STERIS 20C in the presence of 1000 ppm AOAC hard water and 5% calf serum at a contact time of 20 minutes after being inoculated with Mycobacterium bovis (ATCC #35743) and allowing the inoculum to dry at room temperature for 30 minutes. Swab sampling from two surface sites and an elution sample from the lumen showed these device sites to be sterile. However, studies carried out in the absence of the acetyl donors (i.e. positive controls) also showed that the device sites were sterile. The reason for this is believed to be that the organisms were washed off in the rinsing processes. These studies indicate that the efficacy of the process is a combination of both the mechanical removal of organisms as well as the sterilization/disinfection effect of the chemical germicide. This testing demonstrated the efficacy of the combined process in being tuberculocidal with this flexible scope. In prior testing in which M. bovis was evaluated in (Gibraltar Report G-80107, see Appendix) in suspension following 8 hours of simulated reuse of STERIS 20C, 6.98 logs of M. bovis were inactivated in 10 minutes demonstrating that the germicide is tuberculocidal.

IN-USE (CLINICAL) TESTING

The original contract called for the USAMRMC to conduct initial field trials of the sterilant following completion of Phase II. However, per the regulatory requirements published in January, 1992[1] and through discussions with the FDA relative to liquid chemical germicides it was clear that in-use testing would be required for regulatory approval. In discussion with the COR in March, 1994 it was agreed that the device testing typically conducted by USAMRMC would not suffice as clinical trials for regulatory purposes. Thus clinical trials were considered as an additional work element required of STERIS with additional funding from the Army received to carry out this task.

Protocols developed by STERIS and reviewed by the Army's Quality Assurance personnel and agreed upon by two participating clinical centers were employed for in-use testing. The two clinical centers were the University Hospitals of Cleveland and St. Luke's Medical Center of Cleveland. At the University Hospitals of Cleveland four devices were evaluated: vaginal speculum, bougie Maloney dilator, a dental curette, and a dental mirror (University Hospitals of Cleveland Report, see Appendix). Each device was studied five times with the exception of the bougie that was studied three times. The expected microbiological results from the various devices depended upon their use intravaginally (vaginal speculum) or orally (mirror, curette). In almost all cases microbiologic bioburden was undetectable post cleaning which attests to the vigorous nature of the cleaning protocols utilized. No microbiological growth was detectable in any instance post STERIS 20C use.

At St. Luke's Medical Center of Cleveland five rigid surgical related devices were evaluated: Karl Storz cystoscope, hemostat (Pakistan brand), (Storz Metz scissors, Karl Storz camera, and Karl Storz trocar). The results indicated (St. Luke's Medical Center of Cleveland Report, see Appendix) that STERIS 20C effectively eliminated bacterial and fungal organisms. All post STERIS cultures were negative with four exceptions. In one case the sampling tube was probably mislabeled. The organisms isolated in three cultures were likely contaminants of the collection or processing procedures. In one case one of the scissors cultures had a probable plate contaminant since only one colony was isolated on the plate. The same scissors post STERIS culture grew a fungal species that naturally occurs in the environment and is considered a contaminant that was probably introduced during the collection process. Contamination during the collection process was also the probable cause of a Staphylococcus isolated from a hemostat as it was not isolated post clinical use or post cleaning of the device. A growth found on the camera, Candida parapsilosis is a clinical isolate. This sample was most likely mislabelled during collection since there was no growth post patient and post cleaning.

The advantages seen in the clinical setting were ease of use, and disposal. There was a slight odor that was acceptable to most clinicians. Achieving the required water temperature was sometimes a challenge due to differences in the hospital's taps but the thermometer on the jugs simplified that. Undissolved crystals were found consistently in the screening and sometimes interfered with pouring. One batch that was mixed and covered and tested three and a half hours later failed the Clinical Indicator and had to be discarded.

CHEMICAL INDICATOR DEVELOPMENT

In the early months of Phase II preliminary studies were carried out on the STERIS PROCESS chemical monitor for STERIS 20, STERIS's buffered peracetic acid based formulation for use in STERIS SYSTEM 1. These studies showed that this chemistry does not work well with the 20C use conditions and that a modification of the chemistry employed with the existing product should result in a feasible chemical indicator or a new chemistry would have to be identified and developed.

This additional work element was required of STERIS per discussions with the COR in March, 1994 and additional funding requested and received to carry out this task. STERIS worked with its vendor of the chemical monitor for STERIS 20 to accomplish this task. STERIS with the vendor (Serim Research) carried out performance evaluations on various designs of the chemical indicator and developed read instructions on these products to detect the level of peracetic acid at or above the minimum effective concentration of 800 ± 50 ppm. One particular design was selected and three lots were used in the in-use testing at the two clinical centers. Based upon its evaluation in this setting some minor modifications were made in the read instructions.

The vendor has developed protocols for the shelf life and simulated use testing of the chemical indicator and performance testing of the various lots. STERIS and Serim evaluated pre-production lots for selection for stability testing by Serim. For a lot of indicators qualified through performance testing by STERIS and put into stability testing by Serim the strips were noted to show failures at 1 week stored at 60°C and eight weeks stored at 50°C. These studies are continuing.

BIOLOGICAL INDICATOR

As originally proposed STERIS was to assess the use of its current biological indicator for use with the sterilant. Testing was carried out showing that it would be technically feasible to employ it; however, further work was not pursued on this since the FDA guidance for liquid chemical germicides does not require a biological indicator for use of the sterilant.

EVALUATION OF TECHNICAL ISSUES

Following a presentation of the unfavorable microbiological results and technical concerns with 20C it was agreed in a meeting with the Army on March 20, 1996 that the research period for this contact would end October 1. Further it was agreed that STERIS would convene a meeting of experts to critically discuss the technical issues: (1) loss of microbicidal efficacy of STERIS 20C in shelf (2) inadequate microbicidal efficacy of new lots produced and (3) inconsistent or inability to sterilize devices in simulated use testing.

STERIS on April 23, 1996 convened a meeting at its facilities with experts, including two from the Army, to discuss the technical issues. Table 12 lists those attending this meeting. During this meeting a number of issues were discussed as related to the chemistry of the product and microbiological and device testing. STERIS, in review of these discussions, identified the tasks to be completed to respond to the issues discussed and their time lines.

Outlined below are the chemistry, microbiological and device testing tasks, their rationale and the results of the investigation.

Chemistry Task #1 - Develop H_2O_2 assay method.

Rationale: In past testing, assays of peracetic acid (PAA) concentration have been made. Hydrogen peroxide, H_2O_2, is a known antimicrobial agent and may be making a significant contribution to the biocidal efficacy of 20C. Variations in the level of H_2O_2 present may account for some of the variation in 20C's efficacy.

Results: A qualitative and quantitative assay were developed and utilized for testing.

Chemistry Task #2 - Possible PAA assay interferences.

Rationale: The biocidal efficacy of 20C solutions has not been consistent with our expectations based upon their measured PAA concentrations. A question was raised as to the confidence we have in this assay and the possible interferences of the components in 20C in this assay.

Results: There are no noted interferences in the PAA assay from the components of 20C. In particular, there was no interference by hydrogen peroxide with the assay up to peroxide concentrations of approximately 10,000 ppm. We are confident that the assayed PAA values are correct.

Chemistry Task #3 - Produce a builders blend containing only sodium perborate and the buffers.

Rationale: The addition of liquid components to the dry builders blends may have been a source of non-uniformity, especially with older lots. Also, one or more of them could be somehow interfering with the biocidal activity of the 20C solution.

Results: A lot (lot P-13) was produced without liquid components. In comparison to a standard blend (lot P10) produced in the same equipment, lot P-13 gave comparable results in testing (pour density, % active oxygen, and pH). Lot P-13 had a longer dissolution time that was attributed to the absence of surfactant, defoamer, and sequestants. Peracetic acid generation with the various lots show no practical significant differences.

Table 12 Attendees At STERIS Summit On 20C

CONSULTANTS	
William Bradbury, Ph.D. Consultant	Wolf-Dieter Mueller, Ph.D. Hoechst Celanese Corporation
Major Stephen T. Bruttig, Ph.D. U.S. Army	Daniel Prince, Ph.D. Gibraltar Biological Laboratories, Inc.
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Chemistry Task #4 - Produce a builders blend without sodium perborate.

Rationale: By adding the perborate separately at the time of sterilant preparation, the microbiologists will be able to test the efficacy of a variety of perborate levels without varying the level of the other builders components. They will also be able to use this builders lot with liquid PAA.

Results: This formulation was intended for specialized testing in which all components of the builders would be the same as the original 20C formulation and the concentration of perborate could be varied independently. This lot P-14 was shown to be comparable to lot P-10 (20C builders formulation produced in similar equipment) with the exception that the pH was slightly lower. This lot was evaluated with liquid peracetic acid and shown to give a low pH (mean of 4.82).

Chemistry Task #5 - Examine the ratio of perborate to acetyl donors.

Rationale: Data reported by other parties suggests that the optimal ratios for peracetic acid generation may be in the range of 1.8:1 for $NaBO_3$ /TAED and 3:1 for $NaBO_3$/ASA.

Results: The mole ratio of perborate to TAED and ASA in the 20C formulation are 7.20 and 3.57 respectively, and the mole ratio of perborate to the total acetyl donors (TAED + ASA) is 2.39. At these ratios the formulation is not perborate limited.

Chemistry Task #6 - Compare the compounding specifications for all previous 20C builders lots to identify any differences in process conditions.

Rationale: 20C builder lots have been produced utilizing equipment ranging in scale from a kitchen blender to commercial scale 20 ft^3 VEE-blender. There could be differences in the process conditions employed which might account for the difference in efficacy observed between early lots (Exp. - 1, 2, 3 produced in a kitchen blender and lots P-1 and 2 produced in a 10 ft^3 VEE-blender) and the recent lots P-5, 6 and 7 (produced in a 20 ft^3 VEE-blender).

Results: All raw materials used in the Precommercial (P) lots were accepted for use based on compliance with the STERIS specification for each material. All materials were used before their expiration dates (about 5 years in sealed containers). All the original validation testing was completed on the Exp 3 lot and lots P-1 and P-2. In summary, the raw materials utilized in the STERIS 20C lots were stable during their use time, and no significant differences in process conditions were identified.

Chemistry Task #7 - Produce a builders blend without benzotriazole.

Rationale: Testing of this builders blend will enable the assessment of the impact of benzotriazole on the sterilant efficacy.

Results: Lot P-15 was produced and contains all of the standard components of 20C except the sodium benzotriazole. This lot when tested with perborate and the acetyl donors gave comparable results (peracetic acid generation) to the standard blend (lot P-10).

Chemistry Task #8 - Construct a complete matrix of the ages of all raw materials utilized previously.

Rationale: Review of the age of the ingredients will permit an assessment of any sterilant stability or potency concerns.

Results: All raw materials were used before their expiration dates and were stable during their use time and all met STERIS specifications.

Chemistry Task #9 - Develop assay methods for assorted raw materials, including TAED.

Rationale: STERIS has relied upon certificates of analysis for the TAED and periodic analyses of returned samples by the suppliers because it lacked an assay method.

Results: Efforts focused on developing a spectrophotometric assay method for TAED based on a modification of the Hoechst-Celanese titrametric method in order to make more accurate measurements of TAED. Further development and validation of this method is required. During the development program periodic evaluations have been made of the TAED by the vendor. While a decrease in TAED concentration has been shown in shelf life the decreases have not caused significant decreases in peracetic acid concentrations.

Chemistry Task #10 - Investigate cloud points of surfactants and defoamers.

Rationale: The use temperatures yielding maximum detergency for surfactants may relate to their cloud points. Efficient wetting/penetration of device surfaces and features (as well as penetration of spore "clumps") is important to the biocidal efficacy of 20C.

Results: The surfactants used in 20C are the same as those used by STERIS in 20D (a different but similar sterilant recently introduced by STERIS for use at 50-56°C in the STERIS SYSTEM 1™ automated sterilization system). Testing has shown that the present surfactant/defoamer package has a cloud point of 56.5°C which is appreciably above the operating and preparation temperatures of 20C. Even for the warm water preparation of the sterilant, which calls for temperatures up to 50°C, the cloud point for the present formulation is acceptable.

Chemistry Task #11 - Produce builders blend(s) with new (different) surfactants and/or defoamers.

Rationale: If other surfactants and/or defoamers meeting manufacturing and safety requirements and having greater detergency than those of our present formulation could be identified their substitution in the formulation could be made.

Results: Based upon testing per Chemistry Tasks 10 and 13, it is not considered advantageous to change the present surfactant/defoamer package for 20C.

Chemistry Task #12 - Examine effects of mixing and filtration on PAA concentration of 20C solutions.

Rationale: Concerns were expressed that in previous testing that PAA concentrations may not be uniform in the solutions being analyzed and that PAA may stratify in the solution.

Results: No significant stratification of the PAA in solution was detected. Also, there was concern that suspended particles in solution may localize the peracetic acid. Tests with filtration of the solution through 0.2 and 0.45μm pore membranes showed that solutions of STERIS 20C measured before filtration and after filtration did not differ significantly.

Chemistry Task #13 - Conduct surface tension analysis on 20C solutions.

Rationale: Efficient wetting/penetration of device surfaces and features (as well as penetration of spore "clumps") is important to the biocidal efficacy of 20C. Surface tension analyses can be used to evaluate our surfactant package in comparison to other alternatives.

Results: The surface tension of 20C solution was determined to be 33.5 dynes/cm^2 in deionized water and to be relatively independent of the temperature of the solution and concentrations of the surfactant and defoamer. This value of surface tension was lower than all but a couple of surfactants tested. A temperature range of 20-52°C did not affect the surface tension of the surfactant package currently used in 20C. In addition, a change in surfactant concentration in the range of 0.001 to 100 times the presently used concentration, also had no effect on surface tension. Testing with hard water and serum for which most of the D-value, carrier and device testing was carried out gave a value of 30.1 dynes/cm^2 . This small difference in surface energy among the various surfactants does not warrant a change. Also, in light of the considerable experience STERIS has with the present surfactant/defoamers package, a change was not deemed to be warranted.

Chemistry Task #14 - Assay benzotriazole levels in lots P-1 and P-2.

Rationale: Assays of benzotriazole in these aged lots would assess its stability and address differences in the biocidal property changes of these lots in time.

Results: Testing showed that there is a decrease in the assayed benzotriazole levels in lot P-1 and P-2 at 30 months. Assays of lots P-5, P-6 and P-7 showed comparable results to the baseline results of P-1 and P-2. No direct correlation between benzotriazole levels can be made with the microbicidal results from these lots.

Chemistry Task #15 - Examine pH effect on PAA generation and stability.

Rationale: It is known that a higher pH promotes generation of PAA and that PAA stability is favored by a lower pH.

Results: 20C has been formulated to provide initially a pH of about 8 and with generation of PAA this pH drops less than 1 pH unit due to the buffers employed. The addition of an organic acid after the PAA generation has peaked in order to lower the pH and improve peracetic acid stability has shown promising results.

Microbiology Task #1 - Develop a test for qualifying spore lots.

Rationale: Changes in spore resistance to PAA could account for the decline in 20C sporicidal efficacy which have been experienced. A simple method is needed for the evaluation of spores to be used in testing to determine if they are significantly more or less resistant than others used previously.

Results: A test method has been developed and SOP prepared.

Microbiology Task #2 - Document B. subtilis spore lots used in prior 20C testing.

Rationale: Differences in spore resistance to PAA could account for the decline in 20C sporicidal efficacy which has been experienced in the aged lots and lots newly produced.

Results: A review of the spore lots used in testing was summarized.

Microbiology Task #3 - Conduct sporicidal carrier tests (8 hours with simulated reuse) on newly produced precommercial lot P-5.

Rationale: It is possible that problems with our D-value methodology may be falsely indicating biocidal efficacy problems with 20C. Conducting carrier tests will either support the validity of our poor D-value results or call into question our methodology.

Results: The carrier testing confirms the poor D-value results seen with the new preproduction lots P-5, P-6 and P-7. Thirty of thirty B. subtilis and C. sporogenes penicylinders were sterile in testing. All Dacron suture loops of these organisms, however, were not sterile after 20 minute exposure to an 8 hour reuse 20C solution.

Microbiology Task #4 - Research spore resistance issues.

Rationale: Increases in the spore resistance over time could account for the decline in 20C sporicidal efficacy experienced.

Results: As the same spore crop was used for all D-value testing (see Microbiology Task #2) there can be no variance of spore production or its method of storage. For this same spore lot tested at 50°C mean D-values of less than 8 seconds (calculated by linear regression) were found, not suggesting a spore crop resistivity problem. Gibraltar Labs which performed early D-value testing of 20C and on two year aged 20C have shown a significantly higher D-value for the aged samples confirming the reduced microbicidal property of 20C after aging.

As a result of the literature search and communications with peers, the SOP for preparation and storage of a spore suspension has been modified and a protocol has been developed to assess spore crop resistivity (see Microbiology Task #1). In conclusion, increased spore resistivity as a cause of the decrease in observed microbicidal activity of 20C is not likely.

Microbiology Task #5 - Determine D-value using liquid PAA in combination with 20C builders not including perborate.

Rationale: By this testing it was thought that if there is a chemical cause of lower biocidal activity of 20C, it would be isolated to either the builders or the active components (perborate and acetyl donors).

Results: This testing was carried out with commercial PAA (35% concentration) at peracetic acid concentrations of about 1100 to 1260 ppm (which is above the MEC for 20C) and pHs of about 5.5. In light of all other D-value testing on the 20C formulation with modified builders (Tasks 6-15) it cannot be assumed that the long D-values are caused by the 20C builders. The testing did suggest that the cause of high D-values, in light of testing with Nu-Cidex for which shorter D-

values were achieved, was due to the low PAA concentration or higher pH (pH for Nu-Cidex is about 4.25).

Microbiology Task #5B - Determine D-value using liquid PAA (5%) in combination with 20C builders not including perborate.

Rationale: Testing under Task #5 was not conclusive. Because the 5% liquid PAA contains a higher hydrogen peroxide concentration (20% vs 7%) than the 35% liquid PAA used in Task #5, because Nu-Cidex uses 5% liquid PAA, because the literature indicates a synergistic effect of the hydrogen peroxide and because the literature notes that the hydroxyl radical weakens the spore coat and increases the sporicidal effects of other agents, it was decided to test the 5% PAA at a higher use dilution concentration of PAA with builders lot P-14.

Results: For this testing the PAA use dilution concentration was about 2050-2200 ppm and the pH was in the low 4 range (comparable to that of Nu-Cidex). D-values (linear regression) were 25-59 sec. This testing suggested that low pH, high PAA (as with Nu-Cidex), and probably the increased H_2O_2 concentration, were beneficial.

Microbiology Task #6 - Determine D-value of 20C formulation without benzotriazole.

Rationale: The objective of this testing was to isolate the effect of the benzotriazole component.

Results: Lot P-15 was tested with the standard concentration of acetyl donors. The D-values were unacceptably long and comparable to those for lots P-5, P-6 and P-7. It is concluded that the benzotriazole component of 20C has no effect on its biocidal efficacy.

Microbiology Task #7 - Determine D-value with no liquid components in builders.

Rationale: The possibility exists that the liquid components (surfactant, defoamer and metal anticorrosive) of the formulation may have degraded in the aged sterilant samples or be an interference in the microbicidal properties of 20C.

Results: In assessing the results of testing with no liquid components or sequestrants (Microbiology Task #10) or no metal anticorrosive (Microbiology Task #6) and the chemical results of the adequacy of the surfactant/defoamer package (Chemistry Tasks #10 and #13) this specific testing was not warranted and therefore not carried out.

Microbiology Task #8 - Determine D-value with new surfactant package(s).

Rationale: Testing under Chemistry Task #10 and 13 did not implicate the present surfactant package as the cause of the lowered antimicrobial property of 20C and other packages did not appear to offer any significant advantages. Since several surfactants had a lower surface tension than that presently used, D-value testing was carried out with one of the surfactants, UD-50.

Results: D-values were over 100 sec; therefore, the present surfactant package does not appear to inhibit the microbicidal properties of 20C and the selection of an alternative package is not warranted at this time.

Microbiology Task #9 - Determine D-value with varied concentrations of surfactants and defoamers.

Rationale: If the surfactant/defoamer concentration was too low then the wetting and penetration of the sterilant may not be sufficient.

Results: Testing under Chemistry Tasks #10 and 13 did not implicate the surfactant/defoamer concentrations as the cause of the lowered antimicrobial property of 20C nor did testing under Microbiology Task #8 suggest that a different surfactant package would offer any advantage. This variation on the present formulation was not deemed to provide a significant advantage and was not pursued.

Microbiology Task #10 - Determine D-value with ASA and TAED and $NaBO_3$ and buffers only.

Rationale: This formulation leaves out the liquid components as well as the sequestrants from the formulation. Testing of this variant of the formulation permits an assessment of the interference effect of the components excluded. The rationale is that if the D-values with this formulation are short, then the liquid components and sequestrants may be the cause of poor efficacy of the standard formulation.

Results: Testing demonstrated that the D-values were long and in the range of those for the recently produced pre-commercial lots P-5, P-6 and P-7; thus, the liquid components and sequestrants are not causative to the low microbicidal property of 20C.

Microbiology Task #11 - Determine D-value with varied perborate levels.

Rationale: Perborate dissolution produces hydrogen peroxide. Higher levels of perborate should produce higher levels of hydrogen peroxide that should contribute to improved biocidal properties of the formulation.

Results: D-value testing with a 50% higher concentration of perborate showed values of >400 sec. The increased perborate concentration was not, by itself, more efficacious.

Microbiology Task #11B - Determine D-value with 4x perborate and 2x acetyl donors.

Rationale: Testing under Task #11 showed that 1.5x perborate with 1x acetyl donors did not provide sufficient biocidal activity.

Results: To maximize the perborate and peracetic acid concentration 4x perborate and 2x acetyl donors were tested with hard water and serum. For PAA concentration of 2660-2970 ppm and H_2O_2 concentrations of 5000-6000 ppm and at a pH of 8.3, D-values were about 176 sec. Despite the high levels of peracetic acid and hydrogen peroxide (comparable to that of Nu-Cidex) D-values were long suggesting that low pH is a contributing factor to biocidal efficacy.

Microbiology Task #12 - Determine D-value with no acetyl donors (ASA &TAED).

Rationale: This testing should determine the biocidal effect of perborate alone. In light of the extensive D-value testing and the past testing with AOAC sporicidal carriers showing that with the exclusion of the acetyl donors the formulation does not have sporicidal activity, the assessment of the effect of perborate alone was carried out with inoculated devices.

Results: All sites on three devices tested were positive confirming that perborate alone is not sporicidal. This testing supports our previous testing with devices where the positive controls were tested in the absence of the acetyl donors and all sites were positive.

Microbiology Task #13 - Determine D-value with unencapsulated TAED & ASA.

Rationale: It was thought that the TAED encapsulant could be interfering with the biocidal action of the formulation.

Results: D-values were long (about 300 sec) indicating that the TAED encapsulant is not inhibitory to the sterilant's efficacy.

Microbiology Task #14 - Determine D-value with unencapsulated TAED alone, without ASA.

Rationale: This task is designed to isolate the efficacy of this acetyl donor alone.

Results: D-values were long and similar to baseline D-value for STERIS 20C lots P-5, P-6 and P-7 which used encapsulated TAED and ASA. Thus, the use of encapsulated TAED at twice its concentration does not lower the D-values of 20C and therefore has no effect on the sterilant's efficacy.

Microbiology Task #15 - Determine D-value with ASA alone, without any TAED.

Rationale: This task is designed to isolate the efficacy of this acetyl donor alone.

Results: D-values were about 110 sec which were less than the baseline D-values for STERIS 20C lots, P-5, P-6, and P-7; however, the 12D times are still not less than 20 minutes. Thus, the use of ASA alone as an acetyl donor at twice its concentration in the basic 20C formulation has no substantial effect on the sterilant's efficacy.

Device Testing Task #1 - Expose rigid devices for 20 minutes at 50°C using recently produced sterilant lots.

Rationale: D-value testing for 20C at 50°C showed results comparable (low D-values) to those obtained with STERIS 20 and 20D (these sterilants are designed for use at 50-56°C in the STERIS SYSTEM 1 Processor). Two devices (scissors and graspers) which were difficult to sterilize at 20°C with STERIS 20C were chosen for study.

Results: For initial PAA concentrations of about 1100 ppm and pH of 7.4 the devices were not sterilized. Increased temperature did not appear to have a significant affect on the ability of the sterilant to sterilize these devices.

Device Testing Task #2 - Test efficacy of new sterilant lots with reformulated surfactant package at 20°C.

Rationale: Past failures in device testing may have been related to failure to penetrate with the sterilant devices features such as crevices. These failures could be the result of inappropriate surfactants (or concentrations thereof).

Results: Based upon testing in Chemistry Tasks #10 and #13 and Microbiology Task #8 it was decided that the present surfactant package does not appear to inhibit the microbicidal properties of 20C and no new surfactant package was selected. Thus, no device testing on a reformulated surfactant package was carried out.

Through the additional testing and investigations of the 20C formulation the cause(s) for the loss of sporicidal efficacy which was initially experienced with real time aged product in shelf life testing and has since been experienced even with freshly manufactured product has not been identified. Therefore STERIS abandoned development of that formulation and will not make regulatory filings in connection with it. STERIS feels that the original goals and purpose of this work are still valid and the needs for such a product exist; therefore, in the closing months of this contract it has continued its research and development efforts on this product (see Reformulation of STERIS 20C).

REFORMULATION OF STERIS 20C

Based upon the Army's Performance Requirements for a Powdered Cold Sterilant, STERIS believes that peracid generating compounds could best meet the need. Further, STERIS feels that reformulation of the STERIS 20C could be accomplished to satisfy these requirements. For these reasons STERIS continued its research and development on a peracid formulation.

Based upon the testing carried out with liquid peracetic acid solutions and the builders (anticorrosives, surfactants, sequestrants) portion of 20C, variations of the basic 20C formulation were investigated. The published literature has noted the synergistic effect of hydrogen peroxide on the biocidal properties of peracetic acid[16-21]. This is believed related to the hydroxyl radical weakening the spore coat. STERIS was able to obtain a container of Nu-Cidex which is a product produced by Johnson & Johnson and sold in The United Kingdom as a room temperature sterilant to replace glutaraldehyde solutions. The peracetic acid concentration of this product was determined to be near 3000 ppm and the pH was shown to be about 4.25. (Note: Initial marketing reports and some preliminary testing by STERIS indicates that Nu- Cidex is not compatible with some devices, possibly due to its low pH). Based upon chemical analysis this product is believed to use commercial 5% liquid peracetic acid solution. D-values with B. subtilis were about 35 sec at peracetic acid use dilution concentrations of over 2400 ppm (see Table 13).

STERIS conducted D-value tests with the 20C builders absent perborate (lot P-14) and 35% liquid peracetic acid (a concentration used in STERIS 20) at PAA use dilution concentrations of about 1100 to 1260 ppm which are above the MEC for 20C of 800 ± 50 ppm. The pH of these solutions was about 5.5. D-values were relatively long (>200 sec). (See Table 13.)

Commercial 5% liquid PAA contains an appreciably higher concentration of hydrogen peroxide than commercial 35% liquid PAA (Table 14). Since studies with Nu-Cidex (which employs 5% liquid PAA) gave favorable D-values at concentrations of about 2400 ppm and above (Table 13), studies with 20C builders absent perborate and 5% liquid PAA at use dilution peracetic acid concentrations over 2000 ppm were carried out. Some variations in D-values were noted but D-values were less than 60 sec and pHs were about 4.2 (Table 13).

The above testing suggested that increased hydrogen peroxide and PAA concentrations and lower pHs are favorable for sporicidal activity. STERIS conducted testing to isolate the importance of these parameters.

In order to develop the dry formulation, a higher (1.5 times) concentration of perborate (with resultant higher hydrogen peroxide concentration) was tested with the standard 20C formulation (builders lot P-14 with 1.5x perborate and 1x acetyl donors). D-values were high (over 400 sec) showing that higher hydrogen peroxide concentrations (1000-2000 ppm) alone are not sufficient to achieve biocidal efficiency (Table 15).

Table 13 D-Value Testing with Bacillus Subtilis of the Nu-Cidex and 20C Builders with Liquid Peracetic Acid

FORMULATION	TEMP (°C)	pH	INITIAL PAA(ppm)	INITIAL H_2O_2(ppm)	D-VALUE (sec)	ENDPT (sec)
Nu-Cidex	20.0	4.23	2831	N/A	34.0	<180
	20.0	4.25	2426	N/A	35.5	<180
	20.0	4.27	1539	N/A	49.6	<180
Lot P-14+/35%Liquid PAA	20.0	5.51	1259	1000	272.7	>900
	20.0	5.50	1267	1000	258.1	>900
	20.0	5.45	1115	1000	223.7	>900
	20.0	5.42	1094	1000	240.5	>900
Lot P-14+/5%Liquid PAA	19.7	4.42	2204	6000-8000*	24.5	<180
	19.9	4.22	2051	6000-8000*	29.5	<240
	20.8	4.19	2132	6000-8000*	38.1	<240
	19.9	4.20	2137	6000-8000*	41.9	<300
	19.3	4.18	2128	6000-8000*	59.2	>300

+P-14 is STERIS 20C builders minus perborate
*VACUettes Hydrogen Peroxide Test Kit (qualitative)

Table 14 Peracetic Acid Chemical Properties (Supplied by FMC Corporation)

Peracetic Acid	5%	35.5%
Hydrogen Peroxide	20%	6.8%
Acetic Acid	10%	39.3%
Sulfuric Acid	0%	1%

Table 15 D-Value Testing with Bacillus subtilis with Hard Water (1000 ppm $CaCO_3$) with 5% Serum

FORMULATION	TEMP (°C)	pH	INITIAL PAA(ppm)	INITIAL H_2O_2(ppm)	D-VALUE (sec)	ENDPT (sec)
P-5[+] + 1X Acetyl donors	19.3	6.04	978	~2000	215.4	>480
	19.9	6.05	1147	~2000	86.5	>480
P-14[†]/1.5XPB+1X Acetyl donors	20	8.36	1528	1000-2000*	700.2	>900
	20	8.34	1562	1000-2000*	460.9	>900

[+] P-5 is STERIS 20C builders of precommercial lot #5
† P-14 is STERIS 20 builders minus perborate
*VACUettes Hydrogen Peroxide Test Kit (qualitative)

Table 16 **Peracetic Acid, Hydrogen Peroxide, pH and Dissolution Time at Varying Perborate and Acetyl Donor Concentrations at 20 Degrees Celsius**

Perborate Concentration	Acetyl Donor Concentration	[PAA] (ppm)	[Peroxide] (ppm)	pH	Dissolution Time (minutes)
2X	———	---	---	---	10 minutes
2.5X	———	---	---	---	10 minutes
3X	———	40	5300*	9.19	10 minutes
4X	———	24	4500*	9.25	10 minutes
5X	———	16	3500*	9.32	10 minutes
5X	2X	2145	8000**	8.73	incomplete at 1hr.
5X	1X	1048	8000**	8.92	22 minutes
4X	1X	954	7000-8000**	8.99	17 minutes
4X	2X	2017	7000-8000**	8.78	19 minutes
3X	1X	1033	7000-8000**	8.81	16 minutes

All sterilant samples were continuously mechanically mixed for 30 minutes.
The peracetic acid and hydrogen peroxide concentrations above were recorded at one hour.
* These hydrogen peroxide concentrations were measured using a qualitative kit.
** These hydrogen peroxide concentrations were measured using a spectrophotometric assay.

In addition, D-value testing of the standard formulation (lot P-5) at the reduced pH of 6 and PAA concentrations of about 1040 ppm gave high D-values of 87 to 215 sec showing that a lower pH alone is also not sufficient to achieve low D-values with the standard 20C formulation (Table 15).

In an effort to investigate higher perborate concentrations, studies were conducted with perborate concentrations up to 5 times that in the standard 20C formulation and with concentrations up to 2 times the acetyl donors to increase the peracetic acid concentrations. This testing showed that the solubility limits were exceeded at concentrations of perborate of five times and acetyl donor concentrations of two times that of the standard 20C formulation (Table 16). Therefore investigations were concentrated on testing with four times (4x) the standard 20C concentration of perborate. In testing this formulation in the presence of hard water (1000 ppm as $CaCO_3$) with 5% serum without any pH adjustment, the initial pH was over 8 and over 8.5 at 3 hours and peracetic acid concentration peaked at about 2800 ppm and rapidly declined to less than 750 ppm at 3 hours.

In testing of the product with 4 times the standard 20C perborate concentration and 2 times the acetyl donor concentration (referred to also as formulation 42) the pH was above 8, hydrogen peroxide concentrations were 5000-6000 ppm and peracetic acid concentrations were over 2600 ppm (Table 17). D-values were about 175 sec with end-points of over 900 sec. Thus, with maximal hydrogen peroxide concentration based on solubility limits of perborate with the acetyl donors and with peracetic acid concentrations comparable to that of Nu-Cidex, 12D values of less than 20 minutes could not be obtained. This testing emphasizes the requirement of lowering the pH of the 42 formulation.

As noted before, STERIS has received marketing information from The United Kingdom that there are material compatibility concerns with Nu-Cidex and it is believed that they are most probably related to its low pH. Therefore, STERIS conducted studies with the four times perborate and two times acetyl donors formulation referred to as formulation 42 as a function of pH. To adjust pH, a compatible crystalline organic acid was added at 30 minutes after the addition of the sterilant powders, a time after which the peracetic acid is generated at the high pH of the use dilution (higher pH favors PAA generation). Solutions of the formulation 42 made in tap waters at pHs of 4 to 7 were investigated and shown to contain high peracetic acid concentrations (>2000 ppm) over a course of three hours at 20°C. While PAA concentrations decreased in time, the drops in concentration were appreciably lower than those seen with the non pH adjusted formulation.

D-value testing was carried out at 20°C on formulation 42 solutions prepared in hard water 1000 ppm as $CaCO_3$) with 5% serum at pHs adjusted to 4 to 7.5 with an organic acid after 30 minutes of mechanical mixing. Table 18 compares the data for these tests with those conducted on the formulation without pH adjustment. At the 30 minute mixing time and prior to pH adjustments, the pHs of the solutions were about 8.4 and the peracetic acid concentrations were above 2660 ppm. Linear regression mean D-values at near comparable

peracetic acid concentrations were shown to be particularly dependent on pH in the range of 6 to 8.4 (Figure 2). The additional testing done at pH 6 (seven tests were conducted versus two for each of the other pHs studied) confirmed the reproducibility of the test results. The mean D-value at pH 6 was determined to be 32.5 seconds with end-points less than 210 seconds for tests conducted with initial B. subtilis spore concentrations of about 10^6 cfu/ml. This D-value agrees favorably with the conservatively calculated end-point D-value of 31.2 seconds. Thus, the efficacy of the formulation with increased levels of perborate and acetyl donors is pH dependent with lower pHs favoring lower D-values. In the pH range of about 4 to 6 there was no noted effect of pH.

In the preparation of the sterilant by the manual jug shaking method, temperatures of up to 50°C have been investigated in the past. As a preliminary assessment of the effect of this higher temperature on peracetic acid concentration a study was carried out with formulation 42 and the formulation pH adjusted to 4 and 7. In testing with 50°C water the peracetic acid concentrations are appreciably lower than when 20°C water is used and significantly lower for the non-pH adjusted solutions. Independent of the pH of the solution, the hydrogen peroxide concentration did not decrease but increased in time over 8 hours of study.

In summary, the preparation of the solutions with higher temperature could be expected to have lower peracetic acid concentrations. Hydrogen peroxide data indicates that its concentration is not decreasing and is independent of temperature. Because of the concern of the effects of low pH on material compatibility, STERIS chose a pH range of about 6-6.5 to focus its testing on. This pH is low enough on the D-value versus pH curve (Figure 2) and is in the pH range for STERIS use dilution (pH of >6) for which the extensive use (over 10 million process cycles with STERIS SYSTEM 1) has demonstrated its compatibility with a broad range of medical devices.

Because of the concern that peracetic acid generation may be dependent on the water quality, STERIS investigated the effect of water types (AOAC hard water at 1000 ppm as $CaCO_3$, tap and deionized). Testing of formulation 42 at pH6 (referred to as formulation 426) with varying qualities of waters has no appreciable effect on the resultant peracetic acid concentration which was maintained above 2000 ppm over the 6 hours tested.

Table 17: D-Value Testing with Bacillus subtilis of Formulation 42

FORMULATION	TEMP (°C)	pH	INITIAL PAA(ppm)	INITIAL H_2O_2(ppm)	D-VALUE (sec)	ENDPT (sec)
P-14/4XPB+2X Acetyl donors	20.4	8.30	2667	5000-6000*	176.4	>900
	21.4	8.41	2971	5000-6000*	173.3	>900
P-14/4XPB+2X Acetyl donors	20.3	4.11	2600	5000-6000*	41.0	<240
	20.5	4.14	2591	5000-6000*	32.5	<180
	19.6	5.07	2707	~4900	30.1	<180
	19.7	5.05	2594	~4900	32.3	<180
	20.0	6.04	2738	~4500	32.3	<180
	19.2	6.02	2812	~4500	29.3	<180
	19.7	6.04	3017	~4500	33.9	<210
	19.7	6.04	2908	~4500	31.4	<180
	19.7	6.04	2887	~4500	29.8	<180
	19.8	6.03	2738	~4000	38.0	<210
	19.9	6.03	2729	~4000	32.8	<180
	19.6	7.06	2948	~4000	44.5	<240
	19.7	7.06	2891	~4000	40.0	<240
	19.7	7.53	2885	~4800	78.4	>360
	20.0	7.53	2824	~4800	59.1	>360
P-14/4XPB+2X Acetyl donors pH 6; 8 Hour Reuse (8 hrs)	20.1	6.00	1914	4730	38.1	<270
	20.1	5.98	1910	5008	38.0	<270
	20.1	5.97	1914	5105	39.1	<2400
(at 9.5 hrs)	20.1	5.99	1809	4700	37.7	<300
	20.3	5.96	1851	4694	38.9	<300
	20.1	5.97	1824	4797	39.3	<270
(at 24 hrs)	20.1	5.79	1054	4871	N/A	>300
	20.1	5.70	1201	4523	N/A	<300
	20.2	5.78	1152	4841	N/A	<300

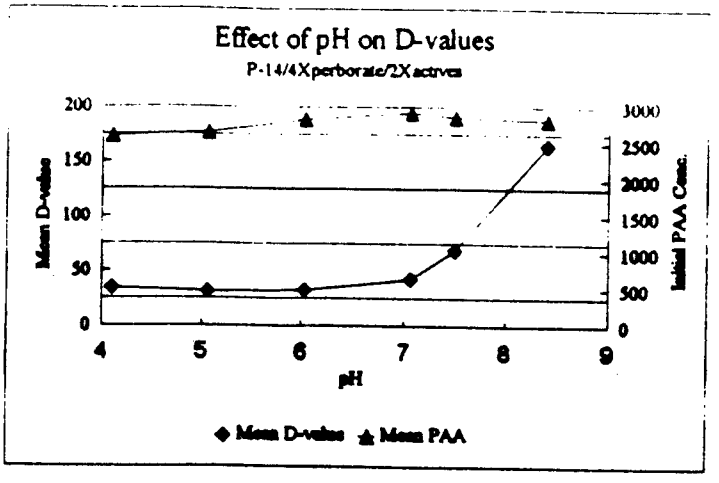
*VACUettes Hydrogen Peroxide Test Kit (qualitative)

Table 18: D-Value Test Data for Formulation 42 at Various pHs

	pH 4 n=2	**pH 5 n=2**	**pH 6 n=7**	**pH 7 n=2**	**pH 7.5 n=2**	**pH 8.4 n=2**
pH AOAC H.W. & 5% Serum (range)	8.03±0.05 (7.99-8.06)	8.06±0.08 (8.00 - 8.12)	8.19±0.06 (8.12 - 8.30)	8.24±0.04 (8.21 - 8.26)	8.07±0.03 (8.05 - 8.09)	8.01±0.17 (7.98 - 8.22)
pH after activation (30 min) (range)	8.38±0.00 (8.38 - 8.38)	8.45±0.06 (8.41 - 8.49)	8.47±0.01 (8.45 - 8.49)	8.43±0.05 (8.39 - 8.46)	8.39±0.00 (8.39 - 8.39)	8.36±0.08 (8.30 - 8.41)
pH after citric acid added (range)	4.13±0.03 (4.11 - 4.14)	5.06±0.01 (5.05 - 5.07)	6.03±0.01 (6.02 - 6.04)	7.06±0.00 (7.06 - 7.06)	7.53±0.00 (7.53 - 7.53)	8.37±0.04 (8.34 - 8.39)
pH after test (range)	4.24±0.00 (4.24 - 4.24)	5.13±0.03 (5.11 - 5.15)	6.09±0.03 (6.07 - 6.13)	7.12±0.02 (7.10 - 7.13)	7.55±0.01 (7.54 - 7.56)	8.38±0.05 (8.34 - 8.41)
PAA before citric acid addition (range)	2967±96 (2899-3035)	2828±171 (2707-2949)	2896±113 (2707-3039)	2859±40 (2830-2887)	2876±116 (2794-2958)	2819±215 (2667-2971)
PAA at start (range)	2596±6 (2591-2600)	2651±80 (2594-2707)	2845±100 (2738-3017)	2920±40 (2891-2948)	2855±43 (2824-2885)	2819±215 (2805-2934)
PAA at finish (range)	2604±83 (2545-2663)	2430±141 (2330-2529)	2658±59 (2582-2739)	2826±55 (2787-2865)	2813±21 (2798-2828)	2607±170 (2487-2727)
Stumbo D-value (sec) (range)	51.2±1.9 (49.8 - 52.5)	65.0±10.5 (57.5 - 72.4)	61.7±10.3 (50.7 - 77.3)	66.6±14.0 (56.7 - 76.5)	109.3±31.9 (86.7-131.8)	209.2±40.6 (180.5-237.9)
Linear Regression D-value (sec) (range)	36.8±6.0 (32.5 - 41.0)	31.2±1.6 (30.1 - 32.3)	32.5±2.9 (29.3 - 38.0)	42.3±3.2 (40.0 - 44.5)	68.8±13.6 (59.1 - 78.4)	165.0±5.9 (160.8-169.2)
End Point D-value (sec) (range)	33.9±6.9 (29.0 - 38.7)	30.3±0.0 (30.3 - 30.3)	31.2±2.4 (29.8 - 34.8)	29.8±0.0 (29.8-29.8)	NA	NA
Endpoint (sec)	<240	<180	<210	<240	>360	>900

Figure 2 Effect of pH on Bacillus subtilis on D-Values:

pH	Mean D-value (sec)	Initial PAA Conc. (ppm)
4.10	34.0	2596
5.06	31.2	2651
6.03	32.5	2833
7.06	42.3	2920
7.50	68.8	2855
8.40	165	2819



This study also demonstrated the importance of pH adjustment as the peracetic acid concentration for the control (not pH adjusted to pH 6) declined rapidly over the 6 hours tested. The addition of the organic acid drops the pH of the solution rapidly and the pH is relatively stable over seven hours tested.

In testing of the formulation 426 for longer periods of time peracetic acid concentrations were over 1000 ppm up to 24 hours and the pH remained about 6 through the 10 hours studied. Hydrogen peroxide data showed fluctuations but were relatively constant in time and above 4500 ppm through 24 hours of testing.

In preliminary testing of formulation 426 the AOAC carriers Clostridium sporogenes inoculated porcelain penicylinders and Dacron suture loop and Bacillus subtilis inoculated Dacron suture loops were tested. (B. subtilis penicylinders were not tested since they did not meet HCl resistance requirements). Five of 30 C. sporogenes suture loops were not sterile while 30 of 30 of the C. sporogenes penicylinders and B. subtilis suture loops were sterile (Table 19). In this testing AOAC procedures were not adhered to due to a methodological error in preparation of carriers and this testing must be repeated.

To assess the formulation 426 with devices, testing was carried out with a scissors, a sheath, and a dental pick. The sterilant solution was prepared in hard water (1000 ppm as $CaCO_3$) with 5% serum and the devices exposed for 20 minutes at about 20°C following inoculation with about 10^6 cfu per site of B. subtilis. The pHs were in the range of about 5.9 to 6.4 and

Table 19 AOAC Carrier Testing with Formulation 426

	HCl Resistance	Enumeration cfu/ml	#Positives/#Tested
B. subtilis			
penicylinders	<2 min.*	1.57×10^2	*
suture loops	>2 min.	1.74×10^2	0/30
C. sporogenes			
penicylinders	$\geq$ 5 min.	1.34×10^3	0/30
suture loops	$\geq$ 5 min.	5.31×10^2	5/30

**B. subtilis* penicylinders did not pass the required 2 minute exposure to 2.5N HCl; therefore these carriers were not tested.

peracetic acid concentrations in the range of about 2100 to 2400 ppm. The dental pick's sites were sterile in five consecutive trials. The scissors' and sheath's sites were not consistently sterilized using this sterilant formulation and method of testing. These devices were previously evaluated with the standard 20C formulation at MEC (800 ± 50 ppm of peracetic acid). This testing showed that the increased peracetic acid and hydrogen peroxide concentrations employed in this testing did not significantly improve on the ability of this sterilant to sterilize these devices.

Additional testing of the scissors and sheath were carried out with the formulation 426 under reuse conditions. In reuse conditions the sterilant is burdened 5 times with B. subtilis at 10^6 cfu/ml. The sterilant was tested at 8 and 24 hours. For the devices inoculated with about 10^6 cfu of B. subtilis per site and for the solution pH of about 5.9 and peracetic acid concentrations of about 1800 ppm at 8 hours and about 1000 ppm at 24 hours, in two trials of each device only one site of the scissors in one trial was shown to be positive for the test organism. This testing at a significantly reduced concentration of peracetic acid compared to that described above does not show a significantly reduced efficacy.

In the course of this testing a jug of Nu-Cidex was received that was three months outside its expiration date. In testing of the scissors and sheath at peracetic acid concentrations of 2422 to 2850 ppm sterilization of these devices could not be obtained. Testing of the scissors and sheath with formulation 42 at pH of 4 showed that they could not be consistently sterilized indicating that the decreased pH did not significantly increase the ability of these devices to be sterilized. From the device testing in which positives were generally seen, the results appeared to be independent of pH, temperature, PAA and H_2O_2 concentrations.

In the investigation of inoculated stainless steel coupons sterilization was achieved with formulation 426. Based on these coupon studies, the results of device testing are believed to be device and device site specific. In some studies on devices in which non-inoculated areas of devices were harvested, recovery of these areas only showed them to be positive. The testing protocols were revised to test only devices that pass sterilization validation criteria when tested in STERIS SYSTEM 1 with STERIS 20 and to have recoverable bioburdens of 10^6 cfu/site with organic and inorganic load (inoculum to be dried on for minimum of 30 minutes). Further, devices were to be cleaned between cycles and if consistent positives are seen on devices they were to be processed in SYSTEM 1 with STERIS 20.

To investigate the feasibility of hand mixing the solutions, jug mixing studies were conducted at varying water temperatures with tap water. The standard protocol for mixing of 20C was carried out. The contents were mixed in the jug for 30 seconds, the jug was then left to set for 15 or 30 minutes and then the contents mixed again for 30 seconds. At the end of mixing, the organic acid was added, mixed and the solution then poured into a pan through a screen and tested. The particulate caught in the screen was dried and weighed to calculate the percent of undissolved powders. The use of higher temperature water results in lower peracetic acid concentrations and less undissolved powders. Also waiting a longer time to add the organic acid results in lower peracetic acid concentrations and less undissolved powders. The undissolved materials were analyzed and shown to be identified as TAED. Based on kinetic studies the setting time for the solution prior to the organic acid addition was set at 15 minutes in future testing.

In light of the formulation differences between formulation 426 and the standard 20C formulation and that TAED is the undissolved material, STERIS investigated the use of ASA alone as the acetyl donor at 4 times its concentration used in the standard 20C formulation. In jug studies with tap water of about 20°C and with waiting only 15 minutes between shaking and the addition of the organic acid, peracetic acid concentrations of over 3000 ppm were achieved and through four hours of testing concentrations of over 3000 ppm were maintained with pHs of about 5.6. Importantly, only an insignificant percentage of the powders were undissolved; in fact, the solution poured readily from the jug.

While prior microbiological testing (D-value testing) of alternative surfactant packages with the original 20C formulation did not sufficiently reduce its D-value to 12D times of less than

20 minutes, one surfactant package in particular did give lower D-values than what was being achieved with the original 20C surfactant package. This new surfactant package was evaluated with the 426 formulation. The concentration effect of the surfactant was evaluated primarily by assessing the surface energy of the solution. No significant differences were seen relative to discoloration, pH, PAA and H_2O_2 concentrations. Based upon the testing the concentration of the surfactant package was selected that achieved the lowest surface energy (about 28 dynes/cm) without effecting the clarity of the solution.

In initial device testing with the scissors and the sheath only one site on the sheath showed positives (this was later determined to be a damaged site). D-values with B. subtilis were in the range of 37-43 sec for PAA concentrations of about 2300 ppm and greater. Preliminary testing with AOAC carriers in the reuse protocol showed some positives; however, peracetic acid concentrations were below 2500 ppm and the HCl resistance on the carriers was greater than 20 minutes indicating that they are very highly resistant. Carrier testing must be repeated.

In continued evaluations of devices all sites on the V. Mueller scissors model SU1733 (3 sites) and the Karl Storz sheath model 27026B (4 sites), five consecutive trials were shown to be sterile for initial peracetic acid concentrations of 2478 to 3055 ppm. In addition four consecutive trials were shown to be sterile for the lumen of the Olympus bronchoscope model P-10.

STERIS is at present scaling up the production of this formulation and continuing the chemical, microbiological and device testing. STERIS is encouraged by its results to date particularly with the device testing.

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BIBLIOGRAPHY OF PUBLICATIONS, MEETING ABSTRACTS AND LISTING OF ALL PERSONNEL RECEIVING PAY FROM THIS CONTRACT

Publications — There were no publications made related to the Contract Work.

Meeting Abstracts — There were no meeting abstracts published nor any presentations made on this Contract work.

Listing of Personnel Who Received Pay From this Contract During its Course

Kathy Bolsen
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STERIS Protocol MS 93-002 - Reuse Protocol for a Medical Device Liquid Chemical Sterilant

STERIS Technical Report T 94-004 - Efficacy Tests of STERIS 20C According to EPA DIS/TSS-9 and DIS/TSS-2 Requirement for Sterilizers: Reuse at 25°C

STERIS Technical Report T 94-005.1 - Sporicidal Efficacy Tests of STERIS 20C According to EPA DIS/TSS-9, Requirement for Sterilizers and DIS/TSS-2, Supplemental Recommendations: Reuse at 20°C

STERIS Technical Report T 94-009.1 - End Point Sporicidal D-Values Using STERIS 20C: Per Reuse at 20°C

STERIS Technical Report T 94-010.1 - Minimum Effective Concentration Determination of STERIS 20C: B. subtilis End Point Sporicidal D-Values

STERIS Technical Report T 94-015.1 - Sporicidal Carrier Efficacy Tests of STERIS 20C at Minimum Effective Concentration at 20°C Following Reuse

Gibraltar Report G-79228 - Determination of STERIS 20C Sporicidal Activity Following 8 Hours Simulated Reuse

STERIS Technical Report T 94-017.1 - B. subtilis End Point Sporicidal D-Value Determination of STERIS 20C at Various Temperatures

Gibraltar Report G-79475 - D-Value Determination on a Variety of Gram Positive Bacterial Endospores Against STERIS 20C

Gibraltar Report G-78632 - D-Value Determination Using Bacteria on STERIS 20C

Gibraltar Report G-79227 - D-Value Determination Using Trichophyton mentagrophytes on STERIS 20C

Gibraltar Report G-78100 - D-Value Determination of STERIS 20C on Poliovirus 2

Gibraltar Report G-78100.1 - D-Value Determination of STERIS 20C on Herpes Type 2 Virus

Gibraltar Report G-79867 - D-Value Determination of STERIS 20C Against Dried Poliovirus 2

Gibraltar Report G-79769 - D-Value Determination Using Mycobacterium bovis on STERIS 20C

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Gibraltar Report G-87871 - D-Value Determination of STERIS 20C Against a Dried Film of Poliovirus 2

Gibraltar Report G-87755 - D-Value Determination of STERIS 20C on Poliovirus 2

Gibraltar Report G-87597 - The Effect of Test Conditions on D-Value Experiments Performed Using STERIS 20C and Bacillus subtilis spores

Gibraltar Report G-80109 - Determination of STERIS 20C Disinfectant Activity Against Staphylococcus aureus , Pseudomonas aeruginosa and Salmonella choleraesuis Following 8 Hours Simulated Reuse

Gibraltar Report G-80107 - Determination of STERIS 20C Tuberculocidal Activity Against Mycobacterium bovis Following 8 Hours Simulated Reuse

Gibraltar Report G-79941 - Determination of STERIS 20C Virucidal Activity Following 8 Hours Simulated Reuse

STERIS Report T 94-016 - Olympus Bronchoscope Type BF P20D, S/N 2900585

Monarch Analytical Laboratories Report COM 94-0-2538 - Test for "Oxidizing And Reducing Substances"

STERIS Report T 94-002 and Addendum - The Results of Quality Assurance Testing of Lots 1, 2, and 3 of STERIS 20C

STERIS Report T 94-007 and Addendum - Quality Assurance Testing of Lots P-1 and P-2 of STERIS 20C

STERIS Report T 94-013 - Laboratory Studies of Pre-Commercial STERIS 20C Shelf Life

STERIS Report T 94-014.1 - Microbiological Effectiveness of STERIS 20C in Storage Under Accelerated Conditions: Bacillus subtilis End Point D-Values At 20°C

Gibraltar Report G-76647 - Acute Dermal on STERIS 20C

Gibraltar Report G-76364 - Primary Dermal Irritation on STERIS 20C

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Gibraltar Report G-77120 - Beuhler Guinea Pig Hypersensitivity Test on STERIS 20C

ABC Laboratories Report 41688 - 96-Hour Toxicity Screen of STERIS 20C to Fathead Minnow (Pimephales promelas)

STERIS Report T 94-021.1 - Analysis of Sterilant Residuals on Instruments Sterilized in STERIS 20C Sterilant

STERIS Report T 94-012 - Materials Evaluations Under Static Continuous Contact With STERIS 20C

STERIS Report T 94-024 - Materials Evaluations Under Static Continuous Contact With STERIS 20C

STERIS Report T 94-044 - Materials Evaluations Under Static Continuous Contact With STERIS 20C

STERIS Report T 94-018 - Evaluation of the Compatibility of Precleaners with STERIS 20C Sterilant

STERIS Report T 94 -011 - Sterilization Study of Representative Medical/Dental Devices Using STERIS 20C at Use Dilution and Manual Soak Technique

Akron Rubber Development Laboratory Report 32918 - Evaluation of Sterilant Permeability

STERIS Report T 95-026.2 - Sterilization Study of Karl Storz Arthroscope Using STERIS 20C Under Simulated Use Conditions With Manual Soak Technique

STERIS Report T 95-027.2 - Sterilization Study of Karl Storz Trocar Using STERIS 20C Under Simulated Use Conditions With Manual Soak Techniques

STERIS Report T 95-028.2 - Sterilization Study of Pilling Microsurgical Tweezers Using STERIS 20C Under Simulated Use Conditions With Manual Soak Technique

STERIS Report T 95-029.1 - Sterilization Study of Storz Microsurgical Scissors Using STERIS 20C Under Simulated Use Conditions With Manual Soak Technique

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STERIS Report T 95-034.1 - Sterilization Study of Pentax Biopsy Forceps Using STERIS 20C Under Simulated Use Conditions With Manual Soak Technique

STERIS Report T 95-035 - Sterilization Study of Medovation Bougie Using STERIS 20C Under Simulated Use Conditions With Manual Soak Technique

STERIS Report T 95-036 - Sterilization Study of Cabot Medical Camera with Coupler Using STERIS 20C Under Simulated Use Conditions With Manual Soak Technique

STERIS Report T 95-037 - Sterilization Study of V. Mueller Microsurgical Hemostat Using STERIS 20C Under Simulated Use Conditions With Manual Soak

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STERIS Report T 95-040 - Sterilization Study of Applied Vascular Angioscope Using STERIS 20C Under Simulated Use Conditions With Manual Soak Technique

STERIS Report T 95-041 - Sterilization Study of Med Peterson Vaginal Speculum Using STERIS 20C Under Simulated Use Conditions With Manual Soak Technique

STERIS Report T 95-042 - Sterilization Study of Circon Cystoscope Using STERIS 20C Under Simulated Use Conditions With Manual Soak Technique

STERIS Report T 95-043 - Sterilization Study of Bard Dilator Using STERIS 20C Under Simulated Use Conditions With Manual Soak Technique

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Gibraltar Report G-87766 - High Level Disinfection of Reusable Flexible Bronschoscopes with STERIS 20C

University Hospitals of Cleveland In-Use Report - In Use Evaluation of STERIS 20C in the Sterilization of Reusable Medical Devices: Evaluation of Devices at University Hospitals of Cleveland

St. Luke's Medical Center of Cleveland In-Use Report - In-Use Evaluation of STERIS 20C in the Sterilization of Reusable Medical Devices: Evaluation of Devices at St. Luke's Medical Center, Cleveland

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REPLY TO
ATTENTION OF:

MCMR-RMI-S (70-1y) 4 Dec 02

MEMORANDUM FOR Administrator, Defense Technical Information Center (DTIC-OCA), 8725 John J. Kingman Road, Fort Belvoir, VA 22060-6218

SUBJECT: Request Change in Distribution Statement

1. The U.S. Army Medical Research and Materiel Command has reexamined the need for the limitation assigned to technical reports written for this Command. Request the limited distribution statement for the enclosed accession numbers be changed to "Approved for public release; distribution unlimited." These reports should be released to the National Technical Information Service.

2. Point of contact for this request is Ms. Kristin Morrow at DSN 343-7327 or by e-mail at Kristin.Morrow@det.amedd.army.mil.

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